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A CRITICAL REVIEW OF THE EVIDENCE ON WHICH IS BASED THE THEORY OF DISCONTINUOUS SYNAPSES IN THE SPINAL CORD

by

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(Submitted for publication 21st July, 1927.)

Despite the researches of several investigators, who have upheld the theory of a protoplasmic continuity at the neurone junctions within the central nervous system, the doctrine of the discontinuous synapse, embracing the body of the nerve cell, or ramifying among its dendrites, has gained an almost general acceptance during the last thirty years. This has been in the main due to the great authority of its main supporter, Ramon y Cajal. In several recent papers I have pointed out that there is but little evidence in favour of this doctrine, but that on the contrary there occurs within the spinal cord a true neurone continuity, not, indeed, a continuity through a superficial Golgi network, as described by Bethe, but a continuity due to the penetration of the collaterals into the dendrites of the nerve cell. The purpose of the present paper is to review the evidence on which the synapse theory is based, and at the same time to discuss the theory of continuity in the form in which it has been presented by other investigators.

Much of the criticism here given of the synapse theory I have already made in previous papers (23, 24, 25); the evidence for the existence of discontinuous synapses within the spinal cord has been so widely accepted, however, and the doctrine is now so generally held, that it seems worth while to take stock of this evidence once more, the more so since a recent reviewer, Peterfi (22), has remarked that the theory of continuity, in the form in which I have stated it, will gain but little acceptance in the face of so much direct evidence for the theory of contact synapses. In this discussion I hope to show that the evidence on which the contact theory is based is, so far as the spinal cord is concerned, extraordinarily scanty.

It will be well to point out at once that the synapse theory is a histological and not a physiological conception. The term synapse was first used by Michael Foster and Sherrington (13) in 1897 for the branching terminations

of neurones, described by Cajal as ramifying amongst the dendrites or embracing the body of a nerve cell *σὺν* and *ἅπτω* (clasp). It is true that the term has entered largely into the literature of a reflex physiology, and that many of the characteristics of reflex action have been attributed to its action.[1] Nevertheless, it is clear that it is entirely a histological idea, and reflex physiology might just as clearly be formulated in terms of any other integrating mechanism in the nerve cells. Consequently, while a study of the neurone junction must have a profound bearing upon the theory of the integrative action of the nerve cell, it cannot play the same part in the theory of the integrative action of the nervous system as a whole.

The outstanding feature of the conception of the reflex arc in the histology of twenty years ago was a protoplasmic discontinuity between its cellular components. The theory of a contact relationship of motor neurone and skeletal muscle fibre has now been replaced by the knowledge that the nerve termination is definitely hypolemmal; even in the case of plain muscle, where Cajal, Retzius, Arnstein, and others found a free termination of nerve endings *between* the muscle fibres, Boeke (6) and Lawrentjew (18) have clearly demonstrated an intraprotoplasmic termination of the nerve fibres. So in the relation of a sense cell to its afferent neurone, Boeke has shown by a series of striking drawings, that the end ramifications of the nerve fibres penetrate into the substance of the receptive cell, frequently terminating near its nucleus (cornea, foliate, and circumvallate papillae, Eimer's organ in the mole, Merkel's corpuscles, taste buds, and Grandry corpuscles); he concludes that the free endings observed by Cajal,[2] Retzius, and others, were due to incomplete staining (5). When protoplasmic continuity can thus be demonstrated at the two ends of the reflex arc, it does not seem probable that discontinuity should exist in its middle. Our present attitude towards the question is inconsistent; thus, while those physiologists who maintain the theory of the myogenic origin of rhythmical contraction of plain muscle tissue look to the protoplasmic bridges between the muscle fibres to transmit the stimulus from fibre to fibre, they do not hesitate to accept the theory of a break in the arc in that very region where conduction might be expected to be most highly specialized, namely, in the central nervous system.

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[1] While the histological bases of most of the component phenomena of nerve integration are quite unknown, there is one, namely fatigue, whose presence can readily be detected. Fatigue of the reflex arc is accompanied by a disappearance of Nissl granules within the nerve cell (Gustav Mann). This observation seems to show quite clearly that in the vertebrate spinal cord, at any rate, fatigue is a phenomenon of nerve cell activity, and accords but ill with the theory that it is a phenomenon of the synaptic membrane situated in close contact with the outer surface of the nerve cell. The observation suggests most strongly that the whole of the integrative processes of the reflex arc occur within the body of the nerve cell, and argues strongly against their occurrence outside the cell. This conclusion is certainly in accord with independent histological evidence described in this paper.

[2] Cajal maintained an interprotoplasmic ending of sensory fibres as late as 1921 (see Cajal, 9). Boeke's results are, however, quite decisive on this point.

Before the introduction of the special methods of Golgi, Ehrlich, and Cajal, the spinal cord was considered, on the basis of Gerlach's observations with carmine stained preparations (1870) to be a diffuse network, the network being formed by an extensive anastomosis of the branching dendrites with one another. The axis cylinders, which had been discovered by Deiters, were regarded as arising by the fusion of outgrowths from the cells of this network and from the network itself.

With the discovery by Golgi (about 1873) of the silver chromate impregnation process, the presence of long conducting fibres within the grey matter was for the first time clearly observed. Golgi failed to confirm the observation of Gerlach of a diffuse network formed by the fusion of dendrites; he found that the dendrites, undergoing extensive branching, ended blindly, and the cell axons were formed as outgrowths, one from each nerve cell. Nevertheless, Golgi still adhered to the theory of a continuous protoplasmic network within the nervous system; he believed that collaterals from the axon of one neurone formed a continuous network with similar collaterals from another neurone, a conclusion which neither Kölliker nor Forel nor Ramon y Cajal could confirm. The diffuse network, he believed, could be specially clearly demonstrated in the spinal cord and cerebellum; but while still maintaining that a continuity between the branching collaterals of two neurones was demonstrable, he held that the close contact between the two systems was enough to transfer the stimulus from one to the other. The diffuse nervous net of Golgi seems to be the dense mass of fine fibrils that are seen pervading the grey matter in very completely stained Golgi and reduced silver preparations.

The main criticism that may be raised against Golgi's conception of the diffuse network is that it is not directly demonstrable. When we observe, in very completely stained preparations (see, for example, figure 4), a dense meshwork of fibrils in the grey matter, it is obviously a matter of mere speculation to regard this as a continuous network; occasional cases of fusion of fibrils may be readily considered as one of the many artefacts observable in Golgi preparations. In reduced silver preparations in which very complete staining has occurred, no indication of a network can be detected. Nevertheless, Golgi appears to have held this view, though in a modified form, even till his death. This attitude towards the synapse theory of the man to whom we are so largely indebted for our present knowledge of the minute structure of the central nervous system shows, at any rate, that the theory of synapses is not based upon such readily demonstrable evidence as we usually suppose. For a statement of Golgi's views his address delivered at Stockholm in 1906 may be referred to (15). This theory of the diffuse network was also upheld by Nansen (21), from studies on the nerve cord of numerous invertebrates, and in addition of Amphioxus and Myxine.

Golgi's theory of the diffuse nervous network was speedily replaced through the work of Cajal, van Gehuchten, Kölliker, and others, by the theory of the discontinuous synapse. By means of the methylene blue process of Ehrlich, and especially of the silver chromate method of Golgi, Cajal was enabled to trace nerve fibres with their finest branches often over incredible distances. These fibres appeared to be of two different kinds: there were those which could be observed to form a pericellular nest on the body of other nerve cells, such as the endings of fibres on the cells of sympathetic ganglia in the frog, or the pericellular terminations of fibres of the acoustic nerve on the cells of the trapezoid nucleus (Held), or the ending of the basket fibres on the Purkinje cells of the cerebellum; and in this group might also be classed the extraordinary neurone junctions occurring in the olfactory glomeruli. The second type of neurone junction was one in which the fibre underwent extensive branching in the grey matter, and as good examples of this type might be taken the branching collaterals in the cerebral cortex and in the spinal cord.[3] It is with this second type that we are especially concerned here. Of the first type it need only be said that the observations of Boeke (5) on the staining reactions of the terminations of nerve fibres seem to show that Cajal's technique in this matter has been very faulty; while, for example, Cajal describes free terminations of nerves upon the surface of the Purkinje cells, Bielschowsky and Wolff (4) find that delicate neurofibrils pass across from these terminations and penetrate the substance of the cell. Marui (20) is able to observe the same thing in the giant Mauthner cell of the fishes' brain. Anybody who has been able to observe how inadequate is the Golgi or Ehrlich method for revealing the intra-protoplasmic termination of sympathetic fibres in plain muscle cells, will lay but little stress upon preparations which show blind terminations of fibres in the central nervous system.

In preparations of the spinal cord, especially of young animals, made by the Golgi technique, the nerve cells have the appearance of enormously branching cells, whose dendrites in some cases may ramify over a very large area of the grey matter contained in the section. As Golgi first stated, and as Cajal and most others have since affirmed, these branching dendrites eventually end free in the grey matter. In some Golgi preparations a considerable number of such nerve cells are to be seen, but relatively few collaterals are stained. It is a curious feature of this method that at other times great numbers of collaterals

[3]It is possible that there is a fundamental difference between these two types of ending. The first type is characterized by the fact that one neurone, without forming collaterals, terminates by an end-ramification upon the body or dendrites of another cell, and a marked convergence of neurones upon this cell does not occur; the second type is characterized by an extensive collateral formation, one neurone terminating on numerous other cells, each of these cells having at the same time numerous collaterals from other neurones ending upon it. While the latter type is specialized for performing integration between excitatory and inhibitory impulses reaching it from different nerves, it does not seem possible to ascribe this property to the first group (the basket fibres of the cerebellum may be an exception to this).

may stain, while the nerve cells in turn remain unstained. In his earlier work (7) Cajal could not satisfy himself concerning the endings of these fibres. But subsequently he concluded, and his views were confirmed by Kölliker and others, that they terminated freely in the grey matter. Occasionally one also obtains Golgi preparations in which a dense meshwork of nerve fibres and also numerous nerve cells have stained. On account of the complexity of the preparations, however, such material is declared by Cajal to be unsuitable for demonstrating the neurone junctions. Cajal's remark is to a certain extent valid; it is certainly extraordinarily difficult to disentangle this dense meshwork with the microscope. The incompletely stained preparations are, however, even less suitable for revealing the relation of collateral to nerve cell; for if only one or the other of these is rendered visible, it is purely a matter of speculation what the relation of one to the other will be. Cajal's general conclusion was that, since in some preparations the branching nerve cells were not visibly connected with nerve fibres (the dendrite branches ending freely), and since in other preparations the branching collaterals always ended freely in the grey matter, there must therefore be a break in the chain, and the neurone junction must be one of contact and not of protoplasmic continuity.

It is clear from these remarks that the theory of synapses, built up as it is from evidence obtained by Golgi preparations, rests upon indirect evidence; in some preparations branching nerve fibres are found to have free endings, while the nerve cells are unstained; in others free nerve cells are found, but branching nerve fibres remain unstained; and it is inferred that the latter end upon the former, the peculiarly selective staining being attributed to a break in the neurone chain.

Although Cajal has given innumerable such illustrations of branching collaterals stained by the Golgi method, ending apparently free in the grey matter, and numerous other illustrations of nerve cells also entirely free, yet, so far as I am aware, he has never observed stained branching collaterals terminating in close contact with the body or dendrites of simultaneously stained nerve cells. For example, if figure 108 of Cajal's book be examined, there is no direct evidence to show that the branching collaterals that enter the grey matter terminate upon any of the stained or upon any of the unstained nerve cells or their dendrites. So far as I am aware there is only one instance in which such a relationship was believed to have been directly demonstrated, namely, that shown in figure 110 of Cajal's book, drawn from a methylene blue preparation, a method which Cajal admits is unsuitable for staining the vertebrate central nervous system. This constitutes the entire direct histological evidence on which the theory of the discontinuous synapse in the spinal cord was originally founded. In addition to these observations there was a considerable mass of indirect evidence in support of the theory. Cajal summarizes this evidence as follows:

(a) The demonstration by His that the neuroblasts of the developing cord are morphologically independent, with the implication that such independence is maintained in the fully developed animal. This evidence loses its force when we consider that a similar independence between a muscle fibre and its motor nerve, observable in the embryo, no longer occurs in the fully developed animal, the motor ending being definitely hypolemmal.

(b) Primary degeneration of a nerve fibre on severing it from the body of the nerve cell does not extend on to the next neurone of the chain. But again it may be pointed out that neither in the case of the motor nerves does it extend beyond the end of the nerve; the ensuing muscular atrophy is a secondary atrophy, and occurs long after the primary degeneration of the nerve fibre is over.

(c) With the methylene blue technique Retzius failed to detect a neurone continuity in the invertebrate nerve cord, an argument which again loses its force if we bear in mind Boeke's warning that this stain cannot always be relied upon to demonstrate the finest nerve fibres (see Boeke, 5).

We see, therefore, that the indirect evidence brought forward by Cajal in support of his doctrine of neurone discontinuity, namely, embryological evidence, evidence obtained from degeneration experiments, and evidence obtained by analogy with invertebrate tissue, is invalid. Let us now examine the direct histological evidence itself.

Silver chromate preparations have led, in the hands of Cajal, Golgi, Kölliker, van Gehuchten, and others, to two conclusions already stated, namely, (1) An independence of the nerve cells, with free endings of dendrites; and (2) A free termination of collaterals that enter the grey matter. Of the latter Kölliker (17) says: "All the collaterals, without exception, end, as it seems, in the same way, namely, with fine end ramifications, which remind us of the endings in ordinary motor end-plates. These ramifications consist of innumerable short branchlets of the finest varicose fibrils, which closely envelop the nerve cells and end usually with fine knobs." In numerous successful Golgi preparations of the nerve cord of newly-born rats and mice I have made the following observations: (1) The dendrites of the nerve cells terminate either by being cut short as they emerge from the section, or they may show an apparently genuine ending. (2) The collaterals end either by being cut short as they emerge from the section, or their ending may be apparently genuine, and these fibres, after undergoing branching, may end in a varicosity, though much more frequently such varicosities are not found. Illustrations of the endings of fibres are given in figures 1 and 4. In such preparations, therefore, the observation of Cajal, and of the other upholders of the neurone theory, can undoubtedly be confirmed. It seems to me, however, that the observation loses its entire point when we consider *that innumerable instances may be found where the nerve fibres on being traced backwards towards or into the white matter, are found also suddenly to end*

without emerging from the plane of the section. In many Golgi preparations, of course, the collaterals can be observed arising directly as lateral outgrowths from the longitudinal fibres of the white matter. But in many highly successful Golgi preparations the relationship cannot be demonstrated. In such preparations the stain does not reveal any connection between the collaterals and the longitudinally running axons from which they have arisen; the observation can

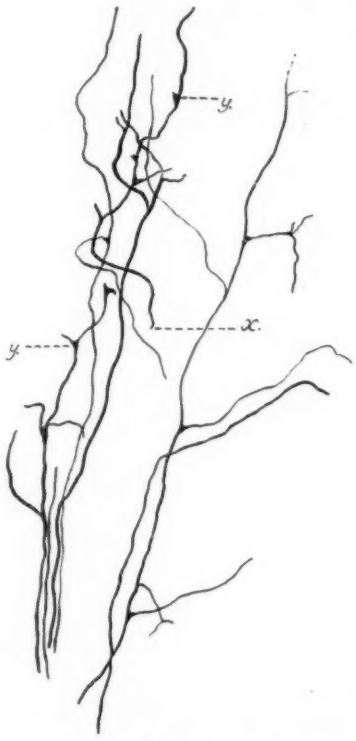

Figure 1. Branching collaterals from spinal cord of new-born mouse, as revealed by the Golgi method. The collaterals, which are taken from a region of the cord adjacent to the central canal, are shown undergoing secondary branching. Most of the collaterals cannot be traced outwards into the white matter, because they are cut short as they emerge from the plane of the section. But at **x** is shown a collateral which, on being traced outwards, ends, not because it is cut short, but because the staining has ceased (the figure cannot, of course, reveal this). The terminations of the finer branches of the collaterals are also not due, in this preparation, to their emerging from the section; it would be unjustifiable to consider them genuine endings or as providing evidence in favour of Cajal's doctrine of terminations by free "end-knobs," since (1) the fibres do not terminate in "end-knobs," and since (2) at some places (indicated by **y**) the staining is obviously incomplete. The illustration is intended to show that even in highly successful Golgi preparations cessation of the staining reaction does not necessarily imply a termination of the nerve fibre.

be made with the greatest ease, and no confusion has arisen with collaterals which have been cut short as they emerge from the plane of the section. We are accustomed to believe that the Golgi technique is capable of revealing collaterals or single nerve cells and all their dendrites in their entirety; these observations show on the contrary that the belief is entirely unjustified. If we once grant, as it seems we must, that the "reazione nera" of Golgi may suddenly cease along the length of a nerve fibre, then we will have to admit that the observation of a free ending of collaterals and of dendrites, upon which Cajal has built the contact theory, cannot be accepted.[4] I need only draw attention here to the recent remarks of Boeke (5) on the extreme difficulty of rendering visible especially the fine terminal parts of delicate nerve fibres; in the case of plain muscle (as has been shown by the use of the Golgi method in Cajal's own hands) it is inadequate.

We have seen, therefore, that the theory of discontinuous synapses in the cord was built up originally on four distinct masses of evidence: (1) embryological, (2) experimental, (3) analogy with invertebrate tissue, and (4) histological; and each of these bodies of evidence we now find is invalid.

The diffuse nerve net of Golgi was, however, so impossible to demonstrate satisfactorily in the cord that the doctrine of a free termination of collaterals on the surface of nerve cells, as first enunciated by Cajal, became generally adopted. On the basis of Cajal's observation Waldeyer in 1891 formulated the neurone theory, a theory which maintains a morphological and physiological independence of neurones in the central nervous system, the neurones being related to one another by contact and not by protoplasmic continuity.

With the subsequent discovery of the reduced silver methods for staining nervous tissue, evidence of a more direct kind was claimed by Cajal for the contact theory; cases were described of nerve fibres ending by "terminal buttons" on the nerve cells, and direct observations of discontinuity in the reflex chain seemed to be apparent. These terminal buttons were held to be identical with similar structures observed by Held (16) and by Auerbach (2), but again we note that these "terminal buttons" were believed by their discoverers to be

[4]Whilst the body of most of the nerve cells in successful Golgi preparations is perfectly free from nerve fibres, one frequently encounters nerve cells with several nerve fibres closely adjacent to the body of the nerve cell. Occasionally one may even observe structures that have the appearance of an end-foot applied to the surface of the cell. Lest it be urged that these fibres are the actual terminations of collaterals, it may be pointed out that there is no evidence in favour of this; some of these fibres are definitely connected with the cell (and are probably collaterals that have penetrated it); others appear to pass over the cell, but their passage cannot be rendered visible, on account of the deep black staining of the cell; fragments of other fibres are also visible, and they may occasionally be seen to "end" in small or larger varicosities, which sometimes lie close to the body of the cell, at other times a considerable distance from it. When we recollect the extraordinary crudeness of Golgi preparations when studied under very high magnifications, we will cease to attribute any significance to these structures; in all probability they are fragments of incompletely stained, delicate fibrils, such as may be observed often in any situation in the grey matter.

definitely fused with the body or dendrites of the nerve cell. They appear, indeed, to be structures first seen by Deiters in maceration preparations, and described by him in his famous posthumous work in 1865. Deiters held that the nerve cells were provided with a single large axon, and numerous very delicate axons which grew out from the body, and dendrites of the nerve cell from minute conical papillae. This free "secondary system of axons" can readily be demonstrated in Golgi preparations, but was considered by Golgi and Cajal to represent merely a branching of the dendrite. I believe, on the contrary that the original idea of Deiters was much nearer the truth than the subsequent opinion of Golgi and Cajal; it can be shown, in fact, that the "secondary system of axons" which Deiters described are in reality the fine endings of collaterals that have grown *into* the nerve cell, and not out from it, as Deiters thought, and that they are the endings of collaterals which Cajal could not observe. It seems to me that the erroneous interpretation of Golgi and Cajal has arisen because the Golgi method is unable to reveal the true nature of the fine fibrils that appear to grow out from the cells and dendrites (but in reality grow *into* them), for it stains the whole cell intensely black, and cannot reveal that these fine fibres are really continued into the nerve cell as its neurofibrils (see figure 4). It is the reduced silver method that can reveal the true nature of these fibres; but it was on the evidence of Golgi preparations that the theory of discontinuity was founded, and the results of the new technique of twenty years later do not seem to have been able to shake it.

With this new technique, however, Cajal claimed, as already stated, to be able to demonstrate the terminal buttons at the ends of the fine collaterals, lying in close contact with the body of the nerve cells. My own results, obtained by this method, are profoundly different; I have not been able to demonstrate the terminal "end-buttons," but on the contrary find that the collaterals grow into the dendrites of the nerve cells, and become continuous with its neurofibrils, an observation which Cajal does not seem ever to have made.

I shall discuss the "terminal buttons" first. By means of the reduced silver technique Cajal has rendered visible what he holds to be the actual terminations of the collaterals, namely, minute cone-shaped swellings at the ends of the fine fibrils, applied closely by their flat surface, to the surface of the nerve cell. For an illustration, figure 112 of Cajal's book may be consulted. In numerous apparently very successful preparations of the nerve cord, stained by this method, I have failed to observe these structures, so that it is difficult to form an estimate of them. We notice in these "terminal buttons," however, that they have never been shown to be connected with undoubted nerve fibres. Can it be that Cajal was observing the endings of neuroglia fibres? It may be pointed out that neuroglia cells may often be seen in close connection with nerve cells, and the endings of neuroglia fibres upon blood vessels frequently, as Cajal himself has found, have this conical appearance. Or can it be, as one so often finds

with adult tissue, that a shrinkage of the cell from its surrounding intercellular matrix draws the latter into fibrils? It must be admitted that it is extremely difficult to obtain an explanation of this figure of Cajal, if one has not been able to obtain similar results oneself; nevertheless, in my own preparations almost every nerve cell seems to exhibit so definite a neurone continuity, though of a very different type from that considered by Cajal, that I find it difficult to accept Cajal's figure as decisive. This much seems certain: that if Cajal is able to observe individual instances of terminal buttons applied to the surface of nerve cells, on the other hand the same method appears to reveal on almost every nerve cell the clearest evidence that the neurones penetrate into the substance of the cell. In any case, we observe in Cajal's own figure that the neurofibril staining has almost entirely failed; consequently he is not able to exclude the possibility of neurofibrils passing from his "end-buttons" into the substance of the nerve cell. One recalls the fact that Bielschowsky and Wolff actually describe such a neurofibril continuity in the terminal buttons which are applied to the Purkinje cells of the cerebellum, and that Donaggio observes the same in the nerve endings on the cells of the Trapezoid nucleus (11), while Cajal in both these situations finds nothing but discontinuity.

Contrary to the observation of Cajal, I find, as described in previous papers (23, 24, 25) that the collaterals which enter the grey matter undergo a considerable amount of branching, especially in the substance of Rolando, and that these collaterals then penetrate almost always into the dendrites of the nerve cells, being continued along these dendrites as the neurofibrils of the cell. Direct proof that the collaterals actually penetrate into the dendrites was at first not forthcoming; it is true that on every nerve cell that had been appropriately cut the neurofibrils of the cell could be observed to emerge from the dendrites and travel as naked neurofibrils in the substance of the grey matter, till they were cut short as they emerged from the plane of the section; and it is also true that the naked neurofibrils are indistinguishable in appearance from the branching collaterals that traverse the grey matter. But direct proof that the branching collaterals were the same as the naked neurofibrils was not at first available, until it was found possible to identify the neurofibrils with the branching collaterals, not by the fact that they arose as collaterals from what could be definitely identified as an axon of another cell, but because collaterals could be recognized in the grey matter by their manner of branching. A branching collateral generally exhibits a small triangular piece of protoplasm where the branch is formed, and the position of this minute piece of protoplasm reveals the forward direction of the collateral. Although, as previously reported (24), the method proved valueless for most of the collaterals which were examined (for even in sections 20μ in thickness they sooner or later emerge from the plane of the section), yet occasionally such a fibre could be followed to its destination, and it was then found to enter into the dendrite of another nerve

cell. Whatever may be said concerning the "terminal buttons" of Cajal, it seems clear that if they really constitute a mode of neurone junction (leaving out of consideration the question whether they exhibit protoplasmic continuity with the cell or not), still the predominant and most easily demonstrated junction is one in which the axon collaterals, after undergoing branching in the grey matter, penetrate the dendrites of the nerve cell and form its neurofibrils. These

Figure 2. A. A dendrite from a spinal cord cell of rabbit, showing its neurofibril organization (reduced silver method). Six neurofibrils are clearly seen "emerging from" the dendrite. The point of "emergence" of two of the neurofibrils is marked by a minute outgrowth from the dendrite.

B. Diagram of portion of the same as it would appear in a Golgi preparation. This would be interpreted by Held as a case of two nerve fibres ending each by an "end-foot" upon the dendrite, the "end-foot" having fused with the dendrite.

neurofibrils then form a perinuclear network, as Cajal himself observed, and from this network a relatively small number of neurofibrils arise and enter the axon of the cell.

If, as maintained above, this constitutes the predominant if not sole type of neurone junction in the cord, it may well be asked why Cajal failed to observe it. So far as I am aware, Cajal never observed naked neurofibrils emerging from the body or the dendrites of the nerve cells. None of his illustrations

ever show neurofibrils emerging from the nerve cells and travelling as naked neurofibrils in the intercellular matrix of the grey matter. On the contrary, Cajal says of the neurofibrils of the nerve cell: In the dendrites "the bundles of neurofibrils thus become finer and finer, till only one remains, very thin, very pale, without varicosities in the core of the dendrite. These neurofibrils do not exist at all at the level of the dendritic spines; or, at least, if they exist they escape our sight and our methods, on account of their paleness and fineness. The neurofibrils which alone have remained in the core of the dendrite can in their turn ramify, as our observations on pyramidal cells have shown; they then end freely, and without a swelling at their ends." Cajal therefore seems to have failed to observe what is apparently the most characteristic feature of the neurofibrils, namely, that they may emerge from the nerve cells and travel for great distances free in the intercellular matrix of the grey matter. An illustration of this point is given in figure 2, drawn from one of many hundreds of cases observed. Numerous other illustrations will be found in my previous papers on this subject. Cajal's failure to make this observation is probably to be attributed to faulty preparations.

Edinger appears, however, to have known this fact, when on p. 25 of his book he remarks: "In addition to ganglion cells, neuroglia and nerve fibres, the grey substance is perhaps composed of numerous branches, which are formed from the nerve fibrils which enter it from the nerve fibres and the ganglion cells. For the vertebrates, the extent to which just this constitutes a component of the central grey matter is still unknown; we believe only that it exists." Edinger nevertheless adopts the conventional idea of the synapse.

In the above discussion it has been argued that while the doctrine of the discontinuous synapse was formulated almost entirely on the evidence of Golgi preparations, yet this method is not only incapable of revealing the penetration of collaterals into the body and dendrites of the nerve cells, but it is also inadequate to establish that very conclusion that has been drawn from it, namely, a neurone discontinuity in the region of the junction. It will now be pointed out that the evidence obtained from Golgi preparations leads to certain conclusions inconsistent with the theory of synapses ending on the body of the nerve cell or ramifying among its dendrites.

In diagrammatic representations of synapses, as they may be found in any text-book, we present a small nerve cell, with relatively short dendrites, ramifying among which are the terminal branching collaterals. The diagram is at first sight convincing enough; but examination of Golgi preparations frequently shows that structures which we call dendrites may frequently ramify over a very extensive region of the grey matter, in which case our diagram becomes difficult to accept. It might be urged against this argument that the small terminal ramification of the axon collateral ends upon a single dendrite only, or ramifies among branches of it; but this would be difficult to correlate with

the fact that sometimes the dendrites that the Golgi method reveals, lie altogether outside the grey matter. For example, in figure 3, taken from Kölliker, it would be obviously impossible to urge a termination of collaterals upon the dendrites of the nerve cell, for the dendrites do not occur in the grey matter!

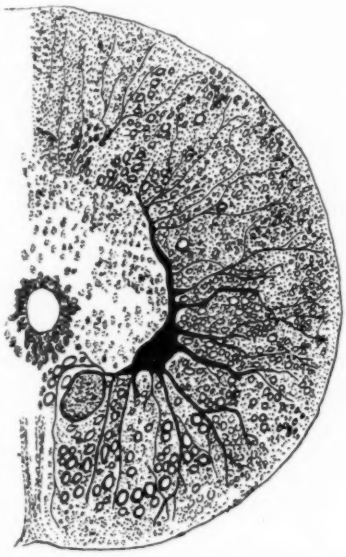

Figure 3. Transverse section of spinal cord of **Protopterus**, after Kölliker.

This might indeed almost be taken as evidence in favour of Golgi's view that nerve conduction was not a property of the dendrites, but that they had a nutritive function. The improbability of Golgi's view is, however, shown by the fact that the dendrites, as revealed by the Golgi method, are, except at their base, structures of extreme tenuity, and the extra free nutritive surface afforded by them must be negligible (see, for example, figure 4).

The objection against Cajal's thesis of the existence of terminal arborizations of collaterals upon the body of the nerve cells of the cord is that they have not been definitely demonstrated. In numerous successful Golgi preparations that I have studied, preparations in which both nerve cells and branching collaterals were sharply stained, I have been unable to find any evidence of nerve fibres converging upon the *body* of the nerve cells. In low-power examination, of course, such a condition may often be seen; we frequently observe a group of nerve cells, with collaterals in the surrounding grey matter showing a tendency to converge upon them. But when we study the same preparations

under high magnifications, we cannot observe any convergence of nerve fibres upon the body of individual nerve cells. For instance, in figure 4 is shown a single nerve cell from the cord of a new-born rat. The cell is surrounded by a dense mass of fibres, but there is no indication that any of these fibres will terminate in a branching synapse on the body of the nerve cell.

There is one case in which Cajal has apparently *directly* observed the application of a terminal arborization to the body of a nerve cell, namely, the case shown in figure 110 of his book. It must be admitted that the figure is extremely

Figure 4. Nerve cell and adjacent nerve fibres from spinal cord of newly-born rat (Golgi method). The figure is intended to demonstrate the fact that, though innumerable fine, branched, and unbranched nerve fibres pass near the region of the nerve cell, none of these terminate by a branching ending (synapse) upon the body or dendrites of the cell, nor do any of them even show a tendency to converge upon the body of the nerve cell. On the other hand, three of the dendrites show clearly fine fibres, indistinguishable from the other nerve fibres, apparently growing out from their substance. But the Golgi method is unable to demonstrate that these fine fibres are continued into the dendrites as its neurofibrils. Only the reduced silver method can reveal the true nature of these fibres. Compare figure 2. Axon indicated at *a*.

convincing, even if it is drawn from a preparation made by the admittedly defective methylene blue technique. My own attempts to reproduce this result have always failed; with methylene blue, spinal cord tissue, as Retzius and others have stressed, stains extremely badly. I find that it presents all kinds of artefacts, among others, heavily vacuolated dendrites, which may assume the appearance of terminal arborizations embracing the nerve cell. But even this cannot account for Cajal's result. It may be pointed out, however, as any Golgi preparation will show, that the collaterals on entering the posterior horn of grey matter, may break into a brushwork of fine branches. In heavily stained preparations these branches, both in Golgi and in reduced silver preparations, may form a dense meshwork in the posterior horn. Can it be that the staining of the branches has ceased in such a manner as to give the appearance of a collateral ending by a branching termination on the body of the posterior horn cell? But whatever the interpretation of this figure of Cajal may be, even if we regard it as a genuine case of a collateral branching upon the body of a nerve cell, it cannot be taken to provide any evidence for nerve discontinuity.

We conclude, therefore, that while the predominant type of neurone junction in the spinal cord is one in which the collaterals penetrate the dendrites of the nerve cell, on the other hand a branching termination of neurones applied to the body of the nerve cell, while it is certainly very difficult to demonstrate, cannot yet definitely be disproved. But even if we admit this latter type of neurone junction to exist, there is no evidence to show that a true protoplasmic discontinuity occurs.

Lest it be suspected that the reduced silver and Golgi methods lead to different results, it may be pointed out that the results obtained by the two methods are quite consistent. While the reduced silver method demonstrates that the neurofibrils may leave the body of the nerve cell as naked neurofibrils in the intercellular region of the grey matter, the Golgi method, failing as it does to reveal any intracellular organization, merely stains the whole cell, with its emerging neurofibrils, deep black. The case is most forcibly illustrated by comparing the results of reduced silver, Golgi, and the average methylene blue preparations. The latter method does not as a rule stain neurofibrils or the great mass of delicate collaterals in the grey matter; on the other hand nerve cells and the dendrites are coloured dark blue. Now in these preparations the dendrites are relatively short, stout structures, do not branch very extensively, and are generally two or three times as long as the diameter of the cell. In successful reduced silver preparations the nerve cell presents the same appearance; in addition, however, the intracellular neurofibrils are sharply stained, and are found emerging from the dendrites and traversing the grey matter for relatively enormous distances, and only terminate when they pass out of the plane of the section. Finally, in Golgi preparations, the nerve cell and its neurofibrils are impregnated deep black, and we gain the impression of nerve

cells with enormously long, delicate "dendrites" travelling far into the grey matter; the greater part of the delicate "dendrite," as the reduced silver method teaches us, is the naked neurofibrils that have emerged from the relatively short true dendrite (or rather, as I have argued, collaterals that have entered *into* the dendrites to become its neurofibrils), and it is only when they are cut short, or when the staining reaction fails along their length, that they appear to terminate (see figure 4).

The doctrine of continuity in the central nervous system has been developed especially by Apathy, Held, Bethe, and Bielschowsky; the theory of the diffuse nervous network of Golgi, as first enunciated, also involved the idea of protoplasmic continuity.

The theory of Apathy (1), as developed by that investigator from a study of the invertebrate nerve cord, maintains that neurofibrils emerge from the trunks of the sensory neurones, traverse the cord as naked neurofibrils, and, after having undergone a certain amount of anastomosis to form the "diffuses Elementargitter," penetrate the motor neurones. Within these they undergo partial anastomosis, and from the anastomosing network a small number (one in the leech) of neurofibrils enter the axon. In my own studies of the vertebrate nerve cord I have not found anything comparable with the Elementargitter, but except for this one point, which may in any case be one of secondary importance, my results are entirely identical with those of Apathy.

Bethe (3) conceived a system very similar to that of Apathy; he observed naked neurofibrils entering the body of the nerve cells, but maintained that these neurofibrils underwent extensive anastomosis on the surface of the cell to form the so-called Golgi net, which he considered to be homologous with Apathy's Elementargitter. This Golgi net, which is often to be observed in methylene blue preparations, was first observed by Golgi in 1893, and regarded by that investigator as a neurokeratin sheath with a skeletal function (14). I am entirely in agreement with Cajal that the Golgi net is a mere artefact, due to the coagulation of the pericellular lymph. If there is any doubt on this point, the figures in a paper by Marui may be consulted (20); it is true that this author holds the Golgi net to be nervous in function, but his figures surely render this interpretation inadmissible. Bethe does not seem to have observed the extensive penetration of the collaterals into the dendrites of the nerve cells, nor did he observe the perinuclear anastomosing network.

Held (16), while considering the Golgi net to be skeletal in function, described the existence of a so-called "pericellular nervous terminal net" on the surface of the nerve cell. He maintained that nerve fibres converge upon the body of the nerve cells, and undergo anastomosis here to form a continuous pericellular network, which has a different appearance from the Golgi net, and is to be looked upon as a different structure. To the argument that the nerve fibres are often found by the Golgi method, in direct connection with the Golgi

network (with the inference that the latter is nervous in function), he replies that the stain has "jumped across" from the nerve fibre to the Golgi net. While this would admirably explain the connection between the nerve fibres and the non-nervous Golgi net, it would render Held's argument for the nervous nature of the "pericellular nervous terminal net" equally invalid.

Both the Golgi net and the pericellular nervous terminal net are, it seems to me, pure artefacts, due to coagulation of the pericellular lymph. I have certainly seen no trace of either in numerous very successful reduced silver preparations.

From the "pericellular terminal nervous net" arise, according to Held, the "end-feet," which bring the "pericellular terminal nervous net" into direct connection with the nerve cell. These end-feet exhibit protoplasmic continuity with the body of the nerve cell.

In his earlier papers Held described a loosening of the "axospongium" of the end-feet, as they fused with the protoplasm of the nerve cell, while at the same time the granules (neurosomes) in the end-feet merged into those of the nerve cell. In later papers he described in the case of the cells of the retina, the Purkinje cells, and the cells of the acoustic and trapezoid nuclei, a direct passage of neurofibrils from the end-feet into the protoplasm of the nerve cell.

It seems to me that the work of Held contains this one important element of truth, namely, that there is a passage of neurofibrils from the end-feet into the nerve cell. The end-feet are, as Held pointed out, the same structures as Deiters observed in macerated preparations fifty years earlier. But while Deiters described them as points from which a secondary system of axons grew out from the cell, Held considered them to be the terminations of fibres *upon* the cell. Structures apparently similar to the end-feet of Held may be observed with ease in Golgi preparations, generally on the cell dendrites. But just as in a previous paper (25) I have argued that the cell dendrite is a protoplasmic outgrowth from the nerve cell which has come to surround a group of neurofibrils that have penetrated into the body of the nerve cell, so it seems to me that the end-feet of Held are to be looked upon as minute dendrites, so to speak, which have come to surround the delicate terminal branch of a collateral as it grew into the substance of the cell (see figures 2 and 4).

SUMMARY.

1. The teaching of Cajal, that there is protoplasmic discontinuity between the elements of a reflex arc has been replaced in recent years by the knowledge that the terminations of motor nerves upon muscle are definitely hypolemmal; whilst the ultimate ramifications of the sensory nerves in the receptive organ are intraprotoplasmic and not interprotoplasmic, as Cajal, Retzius, and others have maintained (Boeke). In previous papers I have described a protoplasmic

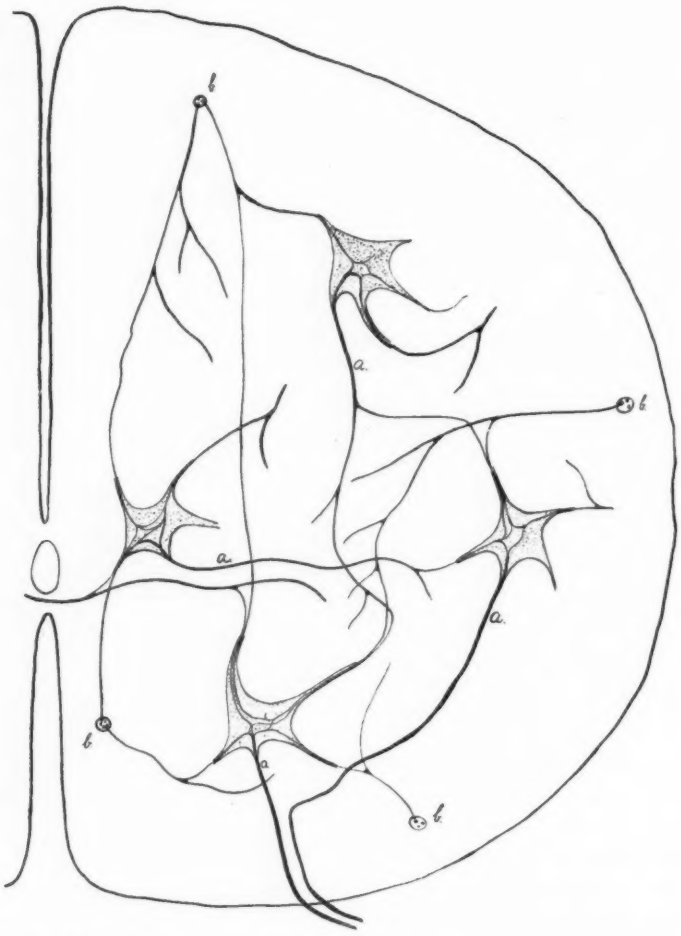

Figure 5. Schematic section of spinal cord, showing relation of collaterals to nerve cells. At **b** are shown four transversely cut, longitudinally running fibres of the white matter, whose neurofibrils are indicated. Collaterals arise by the separation out of neurofibrils (Bethe, Lenhossek). The collaterals branch in the grey matter. Many of the branches are cut short as they leave the plane of the section; others enter the nerve cells, and are recognizable as neurofibrils within the cells. The axons of the nerve cells are represented by *a*; no attempt has been made to represent the neurofibril composition of the axons. The perinuclear anastomosing neurofibril network is indicated in the four nerve cells.

continuity of the neurone junctions within the central nervous system. As the theory of contact synapses has been so generally accepted, the evidence on which this theory is based is here critically reviewed.

2. The theory was formulated by Cajal, Waldeyer, and others upon histological studies of Golgi preparations, and on the indirect evidence obtained from degeneration experiments, and observations on embryonic tissue. It is pointed out that the results obtained from the last two methods apply also to the motor nerve endings on muscle; nevertheless, the motor nerve ending exhibits protoplasmic continuity, and not contiguity.

3. The evidence of Golgi preparations is not decisive; it rests upon the fact that in some preparations axons and their collaterals stain deeply, while the nerve cells do not stain; the collaterals also very frequently end freely. It is pointed out, however, that this free ending of collaterals is valueless as evidence in support of a discontinuity at the neurone junction, since, if we trace the collateral backwards towards the white matter, it again very frequently ends blindly, definitely due to incomplete staining. The Golgi method therefore does not provide a complete picture of the neurone collaterals.

4. Subsequently the evidence for the theory of contact was amplified by Cajal by studies on reduced silver material. In such preparations fine collaterals were described ending in minute "end buttons" close to the body of the nerve cell. Held, on the contrary, found these "end buttons" to be in direct continuity with the cell protoplasm.

5. My own studies lead to the conclusion that the collaterals, which have arisen from the longitudinal axons of the white matter by the separation out of individual neurofibrils (Lenhossek, 19; Bethe, 3), traverse the grey matter of the cord, and after undergoing considerable branching penetrate the dendrites of the nerve cells; they pass as neurofibrils along the dendrites to the middle of the cell, where they form a perinuclear network, which Cajal himself observed, and from this network a relatively small number of neurofibrils enter the axon (see figure 5). The conclusion seems unavoidable that the neurofibril is the conducting unit of the central nervous system. Except for the fact that no anastomosis of fibrils occurs in the intercellular part of the grey matter, my results are identical with those obtained by Apathy on invertebrate tissue.

6. It is pointed out that the evidence from Golgi preparations is not incompatible with this theory. The method has its limitations, because it cannot reveal the internal organization of the nerve cells. The reduced silver method alone is competent to do this, but even with this method Cajal does not seem ever to have observed neurofibrils of the nerve cell emerging from the dendrites and traversing the grey matter as naked fibrils for very great distances.

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THE LIVER GLYCOGEN AFTER PARTIAL PANCREATECTOMY IN THE GUINEA PIG

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(Submitted for publication 18th July, 1927.)

In a recent investigation of pancreatic regeneration in the guinea-pig by G. R. Cameron (1) the opportunity was afforded me of investigating the glycogen content of the liver and the residual content of adrenaline in the suprarenals in a large number of guinea-pigs. This experimental material was divided into three main groups, and each of these into two sub-groups. In the animals of Group 1 (a) a portion of the pancreas had been removed, while those of Group 1 (b) were normal controls from the same litters as the operated animals. In Group 2 (a) the guinea-pigs, like those of 1 (a), had a portion of the pancreas removed, but received a daily injection of one unit of insulin subcutaneously for a period of some months. Group 2 (b) was composed of control animals from the same litters as those of 2 (a), which received the same doses of insulin, but were not operated on. In the guinea-pigs of Group 3 (a) part of the pancreas was removed, and they received daily ·05 c.cm. of pituitrin (Commonwealth Serum Laboratory) per kilo of body weight. Group 3 (b) was composed of control animals from the same litters are 3 (a), which received similar pituitary injections, but were not subjected to operation.

The degree of pancreatic deficiency caused by the operations was not severe, as the sugar tolerance curves obtained during the progress of the experiment showed no abnormality, except in animals of Group 3, which were receiving injections of pituitrin.

The average growth curves of all the groups were normal until the last two months of the experiment, when the injected animals lost some weight, and the dosage of insulin and of pituitrin had to be decreased.

The animals were killed in the same order as that in which they had been put up in cages and operated upon 113 to 314 days from the date of operation, and in the injected animals 89 to 278 days after these were commenced. Control animals were killed as far as possible on the same days as their litter mates.

Methods.

The animals were killed almost instantaneously by dividing the vessels in the neck after producing unconsciousness by a blow on the head. The liver and adrenals were removed and weighed, and the whole of these organs were at once used for the estimations. Glycogen was determined by Cole's modification of Pflüger's method (2), adrenaline by the method of Folin, Cannon and Dennis (3), using the slight modifications described by Kellaway (4). Estimations of the urea in the blood at death were also made in many cases by Maclean's modification (5) of Van Slyke's method.

Results.

The results are shown in Tables 1, 2, and 3. In these the alphabetical letters indicate the litter to which the animals belonged; the body weights are the weights of the animals less the blood shed at death and the contents of the alimentary canal and urinary and gall bladders; glycogen is expressed as percentages of fresh liver weight, adrenaline in mgms. per kilo of body weight, and urea in mgms. per 100 cc. of blood.

Glycogen estimations were carried out in 18 operated animals of the first group, and varied between 0·48% and 6·09%, the mean value being 1·75%. In the control group, among 14 observations the extremes were 0·61% and 3·46%, and the mean was 1·81%. There appeared to be no appreciable alteration in the storage of glycogen in the liver following the removal of a large portion of the islet tissue of the pancreas. In view of the large variations in the value for glycogen the observations are probably too few. The means in the two sub-groups are, however, almost identical; a result which was expected, as the animals which had been subjected to operation were in good condition, and had shown no disturbance of carbohydrate metabolism, giving normal sugar tolerance curves during the course of the experiment. In these animals Cameron found quantitative evidence of the regeneration of islet tissues.

In the second group which received insulin in both operated and control animals there was a diminution in the amount of glycogen stored. This was more marked in the unoperated group, in which the mean value of 23 observations was ·90%, as against 1·32% for 18 animals in which a part of the pancreas had been removed.

The administration of insulin to normal rabbits, both in the fasting and in the fed condition, has been shown by Macleod and McCormick (7) and by Dudley and Marrian (8) to cause marked depletion of the glycogen in the liver. The present observations seem to show that while the insulin administered to these animals stimulated glycogenolysis, it was aided in this action by insulin secreted by the islet tissue. Now Cameron's experiments showed that the

administration of insulin inhibits the regeneration of islet tissue which occurs after removal of a part of the pancreas, and, in agreement with his findings, my results suggest that the output of insulin from the pancreas is less when a portion of the pancreas has been removed and when regeneration is prevented from occurring.

In the third group in which pituitrin was injected the glycogen values were 1·44% in 20 operated and 1·59% in 23 control animals. Whether these lowered mean values, 1·44% and 1·59% as compared with 1·75% and 1·81% in Group 1 are significant, is open to question. The diminution cannot readily be explained by any direct action on the liver, since pituitrin appears to oppose glycogenolysis caused by the injection of adrenaline Stenström (9), and the antagonism between pituitrin and insulin demonstrated by Burn (10) might have led us to expect that in animals in which pituitrin was injected decreased glycogenolysis would have occurred, and would have been reflected in values for the liver glycogen rather higher in these animals than in those of Group 1. Cameron has, however, shown that the injection of pituitrin causes in both normal and operated animals a marked increase in the proportion of islet tissue. It is probable, therefore, that repeated injections of pituitrin in these animals stimulated the pancreas to increased production of insulin, and after the effect of the last pituitary injection had passed off the excessive output of insulin determined an increased amount of glycogenolysis.

The results obtained for the residual adrenaline in the suprarenals are more difficult to interpret, though generally speaking high glycogen values are found with low amounts of residual adrenaline, and *vice versa*. This is not clearly reflected in the mean values, for in some animals glycogen and in others adrenaline was not estimated.

In Group 1 (a), in which a portion of the pancreas was removed, but in which regeneration was found by Cameron to have occurred, the mean value of 18 observations was ·371 mgms. per kilo. The mean value of 10 observations in the control group was ·287 mgms., a result which is almost certainly too low, and must be disregarded, as the number of estimations on which it is based is too small.

In the group in which insulin was injected the mean value of 18 observations on operated animals, Group 2 (a), was ·334, as compared with ·365, the mean of 22 observations in the control group, 2 (b). The influence of insulin in operated animals in which regeneration of the islet tissue has been prevented from occurring (Cameron) appears to have been to cause a lowered residue of adrenaline in the gland, ·334 as compared with ·371 mgms. per kilo in the operated group without insulin (Group 1 (a)). This result, if significant, is very difficult to explain. Cameron, McIvor and Bliss (11) and Houssay, Lewis and Molinelli (12) have shown that the output of adrenaline is increased during

insulin hypoglycaemia. As the last injection of insulin was given in these animals 24 hours before death, it is unlikely that this dose, even if it caused hypoglycaemia, would affect the residual store of adrenaline, and we are therefore concerned only with the insulin normally produced by functioning islet tissue in the two sets of animals. The general inverse relationship between the amount of glycogen in the liver and the adrenaline stored in the suprarenal would have led us to expect a lower residual adrenaline in the operated group, where the mean value of glycogen is higher. Since as long as glycogen is present in the liver adrenaline can cause glycogenolysis and compensate for the lowering of blood sugar caused by insulin, it is conceivable that in these animals the low residual content of adrenaline may be attributable to diminished need for storage when a normal amount of insulin can no longer be secreted.

Finally, in Group 3, in both operated and unoperated animals in which pituitrin has been injected, and in which, as Cameron showed, over-production of islet tissue occurred, the values for residual adrenaline were ·384 and ·387 mgms. per kilo, a finding which is in accord with the hypothesis suggested above.

The estimation of blood urea yielded no significant findings in any of the experimental groups, though it served to indicate the poor condition of animal E.E.2, which was moribund when killed, and in which it reached 177 mgms. In a few other cases in which the animals were apparently in good condition, the blood urea was higher than normal.

CONCLUSIONS.

1. Prolonged daily injections of small doses of pituitrin to guinea-pigs leads to diminution of the glycogen content of the liver. A similar but more marked change, which is to some extent compensated if a portion of the pancreas has been removed, follows the daily injection of insulin. Partial pancreatectomy by itself is without effect.

2. The residual adrenaline in the suprarenals appears to be diminished following the injection of insulin in guinea-pigs in which a portion of the pancreas has been removed, though not in normal animals. Somewhat higher values were obtained following the injections of pituitrin.

3. No significant changes in blood urea were observed in these experiments.

ACKNOWLEDGMENTS.

My thanks are due to Dr. G. R. Cameron for making the materials available for this research, to Miss Cecil Maudsley for help in some of the estimations, and to Dr. C. H. Kellaway for his kindly help and criticism.

TABLE 1.

Partial Pancreatectomy Alone.

Operated: Group 1 (a).						Controls: Group 1 (b).					
Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.	Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.
A1	M	626	0·79	0·279	49	A2	F	667	—	—	—
H1	M	637	0·95	0·342	45	H4	M	641	1·43	0·217	44
H2	M	708	6·09	0·213	43						
X1	M	571	1·01	0·567	63	X2	F	682	1·87	—	—
FF1	M	668	0·49	0·408	65	FF2	M	695	1·20	0·355	55
GG1	M	562	1·18	0·539	45						
OO1	M	533	0·58	0·413	—	OO2	M	660	0·61	0·333	55
PP1	M	535	0·48	0·359	63	PP2	M	688	2·04	—	—
LLL1	M	643	2·08	0·472	48	LLL4	M	830	3·29	0·317	41
D1	F	585	4·33	0·291	54	D2	M	669	1·80	0·267	42
K1	F	742	3·32	0·246	54	K2	F	650	2·41	—	—
S1	F	718	1·70	0·313	45						
V1	F	658	0·60	0·375	48						
V2	F	611	1·11	0·376	48	V3	F	640	1·28	0·301	65
Y1	F	549	0·82	0·453	47						
UU1	F	632	1·49	0·339	22	UU3	F	770	1·07	0·277	58
UU2	F	610	2·98	0·346	30	UU4	F	682	1·14	0·322	—
BBB1	F	624	1·49	0·354	56	BBB2	M	660	2·46	0·172	99
						PP3	M	667	1·33	0·310	37
						O2	F	673	3·46	—	—
Mean	M		1·52	0·399	53	Mean	M		1·77	0·281	55
„	F		1·98	0·344	45	„	F		1·87	0·300	62
Total			1·75	0·371	49	Total			1·81	0·287	56

TABLE 2.

Pancreatectomy and Insulin.

Operated: Group 2 (a).						Controls: Group 2 (b).					
Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.	Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.
B1	M	762	3·62	0·209	40	B3	M	626	0·65	0·309	66
E1	M	599	0·72	0·308	57	E2	F	579	0·80	0·323	49
L1	M	614	1·40	0·236	95	L2	F	585	0·39	0·542	53
HH1	M	654	3·31	0·379	43	HH2	M	633	2·08	0·458	—
MM1	M	638	0·43	0·291	50	MM2	F	691	1·59	0·267	51
RR1	M	704	1·31	0·360	65	RR2	M	612	0·63	0·398	30

Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.	Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.
SS1	M	641	1·33	0·312	37	SS2	M	700	1·26	0·304	55
SSS1	M	667	2·09	0·290	50	SSS2	F	600	0·96	0·383	42
B2	F	682	1·72	0·329	41						
P1	F	553	0·32	0·298	70	P2	M	812	0·22	0·367	46
CC1	F	530	0·99	0·630	55	CC2	F	800	2·13	0·371	63
DD1	F	610	0·58	0·401	52	DD2	F	644	0·59	0·150	64
VV1	F	594	2·08	0·311	—	VV2	F	642	0·85	0·370	37
WW1	F	585	0·64	0·441	55	WW2	F	547	0·94	0·463	40
AAA1	F	636	0·46	0·337	45	AAA2	M	671	0·65	0·345	40
CCC2	F	647	0·49	0·257	52	CCC3	F	529	0·79	0·391	67
DDD1	F	822	0·66	0·243	52	DDD2	M	670	0·79	0·338	—
MMM1	F	608	1·61	0·424	48	MMM4	M	590	0·82	0·415	47
						III2	M	659	0·81	—	—
						AA3	M	680	—	0·360	36
						Z2	F	576	1·04	0·408	56
						AA2	F	680	0·46	0·316	55
						HH3	F	600	0·99	0·436	48
						DDD3	F	635	0·80	—	56
						AAA3	F	720	0·40	0·320	47
Mean	M		1·78	0·298	55	Mean	M		0·89	0·366	46
„	F		0·96	0·364	52	„	F		0·90	0·365	52
Total			1·32	0·334	53	Total			0·90	0·365	49

TABLE 3.

Pancreactectomy and Pituitrin.

Operation: Group 3 (a).						Controls: Group 3 (b).					
Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.	Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.
C1	M	713	1·26	0·314	41	C3	F	428	1·18	—	—
N1	M	500	1·60	0·484	65	N3	M	709	3·03	0·352	56
Q1	M	585	3·75	0·319	—	Q2	M	708	0·37	0·296	55
T1	M	801	0·44	0·337	50						
III	M	695	3·69	0·343	58	112	M	691	2·68	0·340	55
LL1	M	685	0·78	0·363	57	LL2	F	600	1·21	0·423	54
QQ1	M	559	1·10	0·429	62	QQ2	M	570	1·43	0·444	57
KKK1	M	608	1·11	0·404	67	KKK2	M	652	1·42	—	—
LLL2	M	695	1·55	0·328	66	LLL3	F	663	1·53	0·422	30
C2	F	633	0·86	0·350	47						
F1	F	636	0·69	0·388	47	F2	F	666	3·26	0·388	34

Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.	Animal.	Sex.	Weight.	Glycogen.	Adrenaline.	Urea.
S1	F	661	2·44	0·363	56	S2	M	717	1·89	0·374	43
M1	F	730	0·65	0·315	50						
N2	F	495	—	—	—	N4	F	560	3·33	0·426	63
Y2	F	653	1·68	—	—	Y3	F	631	0·60	0·468	49
BB1	F	453	—	—	—	BB2	F	720	0·43	0·377	60
EE1	F	569	0·71	0·522	60	EE2	F	524	0·50	0·103	177
XX1	F	534	0·55	0·491	57	XX2	F	550	0·66	0·434	71
YY1	F	650	2·30	—	—	YY2	F	641	0·69	0·369	61
EEE1	F	785	1·41	0·303	35	EEE2	F	662	0·88	0·374	44
FFF1	F	671	1·15	0·399	38	FFF2	F	610	1·27	0·371	56
MMM2	F	579	1·11	0·464	56	MMM3	F	560	1·49	0·408	47
						YY3	M	680	1·57	0·387	44
						KKK3	M	681	3·48	0·440	49
						HHH1	M	649	3·14	0·468	66
						G1	F	672	—	0·513	63
						TT2	F	611	—	0·364	—
						EE3	F	629	0·71	0·349	71
Mean	M		1·70	0·369	58	Mean	M		2·11	0·388	53
„	F		1·23	0·399	49	„	F		1·27	0·387	53
Total			1·44	0·384	54	Total			1·59	0·387	60

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A SIMPLE SPECTRO-COLORIMETER

by

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(From the Walter and Eliza Hall Institute, Melbourne).

The instrument to be described was constructed for the rapid optical estimation of substances whose solutions show well-defined absorption bands in the visible region of the spectrum, in the presence of other substances having a less selective absorption in this region. Such estimations are usually carried out on a spectro-photometer, but its cost and complexity place it out of the reach of many smaller laboratories and institutions. During the initial experiments on globin, carried out by Hill and Holden (1), an approximate estimation of the concentration of globin in the solutions was made by adding to such a solution an excess of haematin in dilute sodium carbonate, reducing the resulting methaemoglobin to haemoglobin, and oxidizing the latter to oxy-haemoglobin with atmospheric oxygen. A solution of oxy-haemoglobin of known concentration was then diluted with known volumes of water until the intensity of its α absorption band appeared to equal that of the α band of the resynthesized oxy-haemoglobin. The comparison was made in two similar test-tubes, using a small hand spectroscope. Mr. Hill suggested that a colorimeter might be substituted for the two test-tubes, and a few preliminary trials were made. When the author left Cambridge it was decided that he should construct a suitable apparatus and endeavour to determine its applicability.

The metal stand of the apparatus was kept as simple as possible, in order that the cost might be as low as was consistent with efficiency. It consists of a cast-iron base, into which are screwed two upright iron rods, which carry simple adjustable screw clamps for holding the spectroscope, lens, and prism. The colorimeter is fastened to the base with clips. The optical parts comprise a Bausch and Lomb 40 mm. biological colorimeter with solid prisms, a 50 mm. microscope objective screwed into a short length of brass tube, a direct-vision spectroscope, and a 25 mm. right-angle prism with its reflecting face silvered. The source of illumination is a 40 w. gas-filled electric lamp with an opal globe, placed about 25 cm. from the colorimeter mirror in a large lamp-house, fitting closely to the front of the colorimeter, in order to exclude stray light. The eye-piece of the colorimeter is removed, and the image of the line between the two fields of the instrument focussed by the objective on the slit of the spectroscope. The right-angle prism is placed between the objective and the spectroscope to enable the latter to be in a horizontal

position, the person using the instrument to remain seated. If the slit be vertical, two spectra are seen, corresponding to the two fields of the colorimeter. In using the instrument the "standard" cup is so adjusted that the chief band in the corresponding spectrum is of a convenient intensity. The "unknown" cup is then moved until the same band on that side equals in intensity that on the "standard side." The calculations are the same as for an ordinary colorimeter. In the photograph the lamp-house has been removed, in order to show the other portions more clearly.

Three examples may indicate some of the uses of the spectro-colorimeter.

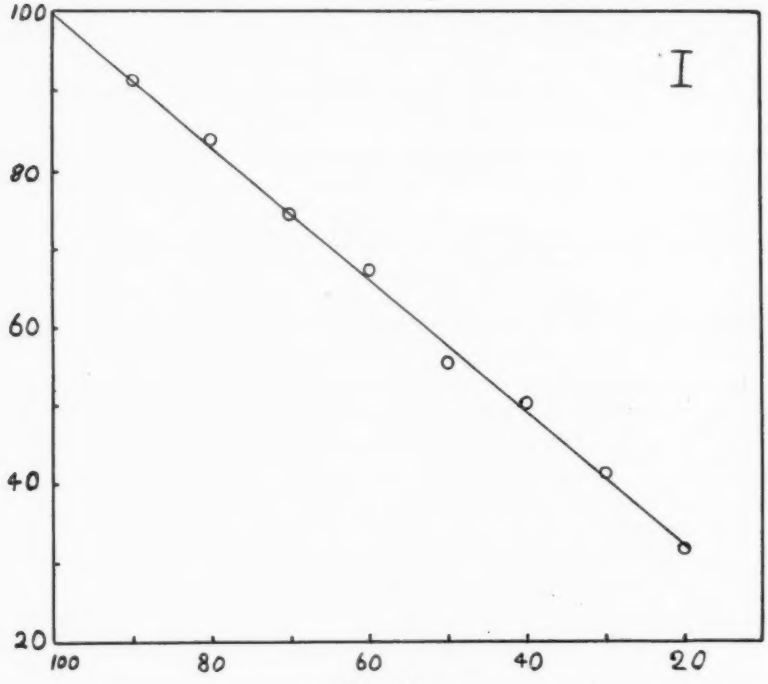
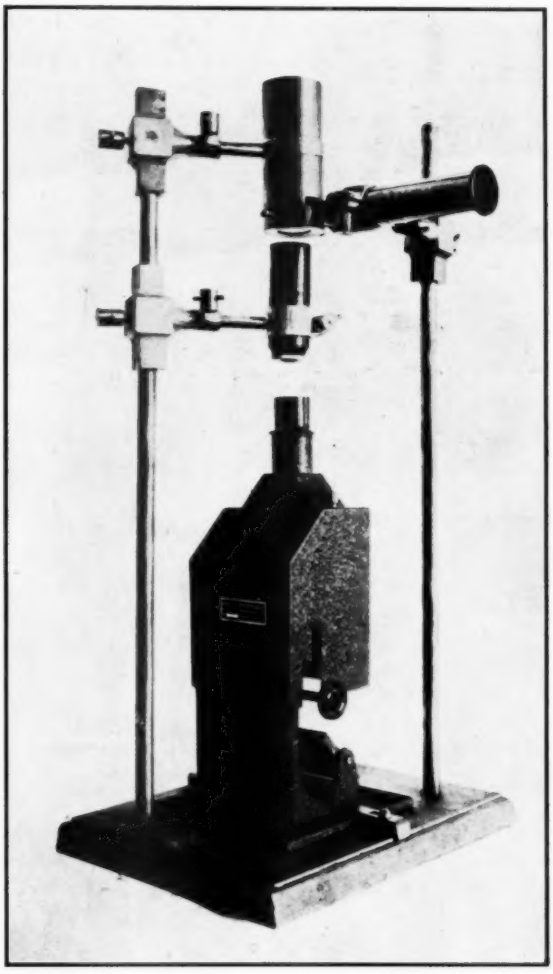


Figure 1.

Ordinates. Apparent amount of oxy-haemoglobin present.
Abscissae. Percentage of pigment present as oxy-haemoglobin, the remainder being alkaline haematin.

From a dilute solution of oxy-haemoglobin a series of other solutions of known relative concentration were prepared by dilution with water. The concentration of oxy-haemoglobin in each was then estimated relatively to that of the stock solution by comparison in the spectro-colorimeter. The results obtained follow:



Actual	..	100	83·3	71·4	62·5	55·6	50·0
Estimated	..	100	83·3	71·9	62·9	56·2	50·2

In such operations as the gradual denaturation of oxy-haemoglobin, where the total molecular concentration of the pigment is known, the proportion which is combined with globin may be determined by applying the correction for the effect of the free haematin present derived from the curve in figure 1. This curve was obtained by estimating the apparent concentration of oxy-haemoglobin in a series of known mixtures with alkaline haematin, so chosen that the total molecular concentration of pigment remained constant. An arbitrary standard of pure oxy-haemoglobin was used.

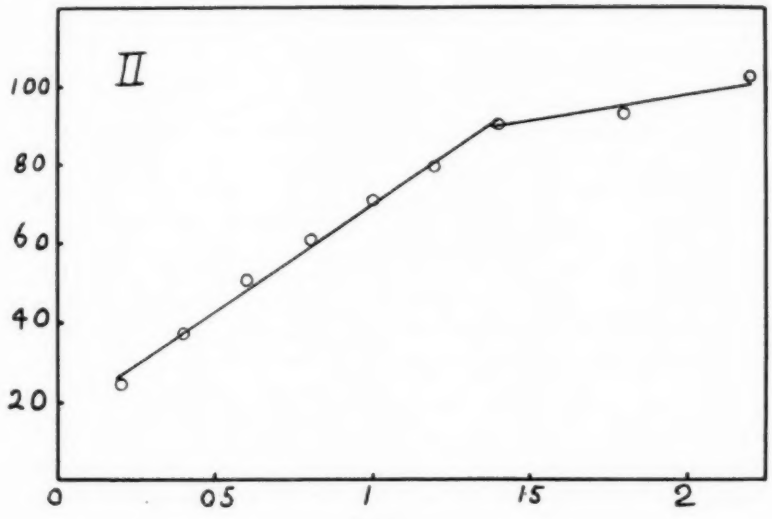


Figure 2.

Ordinates. Oxy-haemoglobin, estimated in arbitrary units.
Abscissae. c.c. of alkaline haematin added. 1 c.c.=0.356 mgm. of haemin.

The instrument may also be used instead of the spectro-photometer for the estimation of the concentration of globin in a solution by titration with haematin (1). Known weights of haematin in sodium carbonate solution are added to suitable measured samples of the globin solution. The pigment is reduced with Stokes' reagent and oxidized by gentle aeration. The amount of oxy-haemoglobin so obtained is estimated against a fixed standard. The discontinuity in the curve in figure 2 marks the point where sufficient haematin has been added to combine with all the globin.

In a few cases the spectro-colorimeter has been employed for the comparison of the intensities of the absorption bands of the spectra of haemochromogens. Many of these compounds, however, form insoluble aggregates, the suspensions of which are unsuitable for spectro-photometric work.

Part of the apparatus was purchased with a grant from the Government Grants Committee of the Royal Society, to whom the writer's thanks are due.

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THE BASAL METABOLIC RATE OF STUDENTS IN SYDNEY, N.S.W., WITH A DISCUSSION ON METHODS OF DETERMINING BASAL METABOLISM

by

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(Submitted for publication 1st September, 1927.)

During the past ten years a considerable amount of data has been collected and published concerning the so-called basal metabolic rate of the body. The greater part of this work has been carried out in America, and the normal standards used for comparison have been worked out for American people. It was thought that it would be interesting to see how the standards would apply in Sydney, New South Wales. The basal metabolic rate has been determined on the students attending the third year course in Medicine at the University of Sydney, and from the results obtained it was found that, using the Du Bois standard for comparison, the average basal metabolic rate was 8·9% lower in men and 10·5% lower in women. Using the Harris and Benedict standard for comparison, the average basal metabolic rate was lowered by 7·6% in the men and 8·6% in the women.

Method.

The method used for the determination of the basal metabolic rate was the open circuit or gasometer method of indirect calorimetry. With this method the subject inspires atmospheric air, and the air expired in a definite time is collected in a Douglas bag. The volume expired is measured, and a sample is removed for the determination of its oxygen and carbon dioxide content. The percentage of oxygen absorbed is then calculated, and the respiratory quotient from which the heat production for 24 hours can be determined.

Preparation of the subject.

By the basal metabolic rate is understood that level of metabolism of the body when the individual is in a post-absorptive condition, in complete muscular repose, and after all exciting influences have been removed. The post-absorptive condition is considered to be reached 12 hours after food, and complete muscular repose is obtained by a preliminary rest of half an hour.

The experiments were carried out during the Lent and Trinity terms of 1926, that is, during April, May, June, July, and August; mostly at 11 a.m. and

12 noon; a few were earlier, namely, at 9 a.m. and 10 a.m. Since the determinations were carried out during the autumn and winter weather, it was considered advisable for students whose basal metabolic rates were not being determined until 11 a.m. or 12 noon to be allowed a light breakfast, rather than ask them to travel anything up to one and a half hour's journey to the University without taking any food at all. Subjects were therefore allowed a cup of tea and two slices of toast for breakfast, which would be taken about 7.30 or 8 a.m. Benedict (6) has shown that a light breakfast of 250 calories, provided it contains little or no protein, increases metabolism by about 2% two hours after ingestion. Soderstrom, Barr and Du Bois (34) allowed their subjects a breakfast consisting of a slice of bread, 8 grms. of butter, one cup of caffein-free coffee, 10 grms. of sucrose, and 66 cc. of milk = 222 calories. This gave an average increase of 7% in the first hour and 2% in the second and third hours in the metabolism of the body.

The question whether the true basal metabolic rate can be obtained on subjects at 11 a.m. and 12 noon, after the muscular movements and activity incidental to the usual routine of a morning's work needs consideration. Benedict and Crofts (7) found practically no increase in basal metabolic rate when measured after rising, bathing, dressing, walking in winter weather for ten minutes, climbing three flights of stairs, and then resting for thirty minutes in a warm room, over that measured before rising, with the body well covered, warm, and relaxed. This finding, they claim, justifies making basal metabolic rate experiments after one-half hour's rest, when it is necessary for the patient to leave home and travel to the laboratory or hospital. Barach and Draper (2) also studied this question, and they published results on a series of patients in hospital and on out-patients who had walked to the hospital, and had thus been exposed to changes of weather and clothing. Provided basal conditions were rigidly observed, no variation in the basal metabolic rate could be detected as a result of the movement, etc., required to reach hospital between these patients and those who had slept at the hospital and had not risen.

The basal metabolic rate of subjects 50 and X, determined at 6.30 and 7 a.m., before rising, and those determined at later hours in the day, at 10 a.m. or 11 a.m., after travelling to the University, show no appreciable difference.

Subject X.		Subject 50.	
Time.	B.M.R.	Time.	B.M.R.
6.40 a.m.	1,057 cals.	7 a.m.	1,411 cals.
6.30 a.m.	1,031 cals.	10 a.m. to 12 ..	1,408 cals. (average)
10 a.m. to 12 ..	1,046 cals. (average)		

From these figures we can take it that the basal metabolic rate of subjects determined at 11 a.m. and 12 noon was not appreciably increased by the usual routine of the morning's work since they rested quietly for one half-hour before the sample of expired air was collected.

Apart from food and muscular movement, the most important factors which can affect the basal metabolic rate are a rise in body temperature and nervous stimulation. It is well known that fever is attended with an increased metabolic rate, and Du Bois showed that this increase in febrile diseases follows van Hoff's law of velocity of chemical reactions with change of temperature. Du Bois showed that since the velocity of chemical reactions increases between two to three times with a rise of 10°C., then a rise in body temperature from 37°C. to 40°C. increases the basal metabolic rate from 30% to 40%, or for every 1°F. rise of body temperature above normal an increase in basal metabolic rate of 7·2% takes place. The mouth temperatures of a large number of the subjects of this investigation were recorded, and there was one record of a temperature above 99°F.

The degree of nervous excitement is, of course, much more difficult to estimate, or even guess at. The room in which the experiments were carried out contained two beds, on which the subjects rested, lying quietly for the necessary half-hour. A fire, to keep the room comfortably warm, was lighted on the cooler days. One realizes that it is a question how much lying down, apparently still, can be taken as a criterion of complete rest and muscular relaxation, and this factor applies equally to patients in hospital as to subjects of this investigation.

The pulse rate of a number of subjects was recorded after the half-hour's rest, and in some cases again ten minutes after commencing the expiration into the Douglas bag. It was thought that the pulse rate would be an indication of the state of rest, that if a subject was nervous or worried by the procedure the pulse rate would probably be increased during the collection of the sample of air. No clear correlation could be established from the results obtained between pulse rates and the basal metabolic rates.

Benedict (3) in 1914 claimed that there is a clearly established relationship between the pulse rate and total metabolism of an individual, and that a reduction, under basal conditions, of 10 beats per minute in pulse rate is associated with an appreciable reduction in basal metabolic rate.

In Tables 3 and 5 a number of basal metabolic rates and pulse rates on one subject are recorded, and it can be seen that a lowering of the pulse rate by 10 beats per minute does not necessarily coincide with a reduction in the basal metabolic rate. These results agree with those of Blunt and Dye (8), who found no relation between minimum pulse rate and basal metabolic rate, whether on the same individual or on a number of individuals.

The days of minimum pulse rate do not always coincide with the days of minimum basal metabolic rate, and certainly persons with a low pulse rate do not have the lowest basal metabolic rates as compared with the figures for their standard metabolism. However, in the later experiments a record of pulse rate was made after the half-hour's rest, just before the air sample was collected,

and again during the collection of the sample, ten to twelve minutes after commencing breathing into the bag. If these two pulse rates do not agree it suggests that the subject is worried by the procedure, and the unusual condition of breathing through the mouth for some time; this indicates that a true basal sample is not being obtained. The results obtained by recording these two pulse rates did not, however, give any information, since in only two cases did the second pulse rate differ from the first by more than 5 beats per minute, and the basal metabolic rate in these two cases agreed well with the determinations made when the pulse rate did not alter appreciably. Still, when determining the basal metabolic rate of patients the record of these two pulse rates may serve to give information about the nervous condition of the patient.

The basal metabolic rate was determined at least twice on each subject, and if the results did not agree within 10% of each other, a third determination was carried out. The determinations were carried out a week apart, the subjects being given a definite time on a certain day of the week, and kept that time for three weeks.

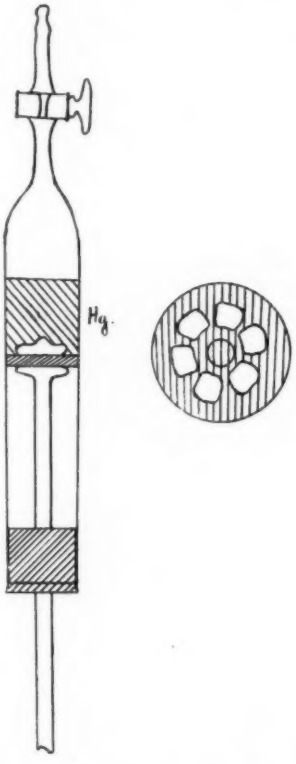
In some cases it was noted that the first determination was higher than the following determination. This has been considered as probably due to a slight nervousness and the unnatural procedure of breathing through a mouthpiece. These factors tend to cause abnormal respiration with irregular rhythm. Associated wtih this there is usually a quickened respiration rate and a higher volume output of air. When the basal metabolic rate is determined a second and third time these factors are greatly decreased, the subject becomes used to the procedure and the apparatus, and breathes more normally and relaxes more completely. Subjects themselves have remarked after the second and third experiment that they felt more at ease that day while giving the sample, and that they expected a truer basal figure would be obtained.

Apparatus consists of a nose-clip, mouthpiece, Douglas bag, and tubing. To the metal box of the mouthpiece were attached two pieces of non-collapsible rubber tubing. Near the junction of each tubing with the mouthpiece was placed a valve, one being an inlet the other an outlet valve. The valves were made of thin sheet rubber, which fitted on flat to a nickelled disc, which had been perforated as in the diagram. The two valves were exactly the same, but reversed in direction. The valves were tested by placing a lighted match at the free end of the tube and breathing through the mouthpiece; if no movement of the flame was obtained at the end of the tube attached to the inlet valve when an expiration was made the valve was considered airtight. Similarly the outlet valve was only considered true if no movement of the flame at the free end of its tubing was obtained during inspiration. If the valves did not stand this test they were discarded.

The tubing attached to the inlet valve was left open to the air, so that the subject breathed atmospheric air. The free end of the other tubing was attached to a Douglas bag of 130 litres capacity. A tap was attached to the tubing near

the bag, so that the expired air could be directed into the room, or by turning a tap, into the Douglas bag.

The subject, after adjusting the mouthpiece, was allowed to breathe through it for five minutes before the expired air was directed into the bag; this was done to give the subject time to get used to the mouthpiece and for the respiration to become regular.



For convenience in calculation the air expired was collected for a period of 14·4 minutes, since this is exactly $\frac{1}{100}$ of 24 hours, the timing being done by the operator by means of a stop watch.

As soon as the sample was collected the bag was well shaken to mix its contents thoroughly, and the air was passed through a dry meter. A thermometer was placed in the meter to record the temperature of the air, and the barometric pressure was recorded at the same time. When about half the contents had been expelled from the bag, a sample of the expired air was collected

by means of a side tube, placed in the tubing between the tap and the bag. The sampling tube was of the nature of a syringe, consisting of about 6 inches of 1-inch glass tubing, provided at one end with a glass tap, and fitted with a glass plunger with a rubber disc. About 1 inch of mercury was placed in the tube above the plunger to act as a seal; the whole was well lubricated with glycerine (see diagram).

When the tube was filled with the air sample the tap was also sealed with mercury. These sampling tubes which held 66 cc. to 80 cc. of air, were quite air-tight, and by simply raising the plunger, so that the tap and its tube were filled with mercury, connecting the tap to the side tube of the bag, and then drawing down the plunger, a sample of the expired air was easily obtained. After connection to the bag the plunger was raised and lowered several times, drawing the air from and expelling the air into the bag and tubing, so a very well mixed sample of the expired was obtained. This type of sampling tube was found to be much simpler to use than the old Hempel tube, with its necessary mercury reservoir. The sampling tube was designed by Sir C. J. Martin, Director of the Lister Institute, London.

The meter used was an ordinary dry meter, and by being placed in series with a standard wet meter, was tested (1) by driving air through the two meters by means of a Crowel blower, and (2) by filling a Douglas bag with air, and then connecting the bag to the meters and expelling the air from the bag by pressure at the rate that is used in measuring the volume of air expired after an experiment. The maximum error of the meter was found to be 2%; the average error was less than 1%.

The analysis of the expired air was carried out by means of a large Haldane apparatus. The apparatus was calibrated by numerous analyses of atmospheric air, and every few weeks check air analyses were performed. The error allowable in the analyses for duplicate figures was CO_2 0·04%, and O_2 0·06%.

As far as possible the air samples were analyzed on the day they were collected, but sometimes they were allowed to stand till the next day, and in a few cases the samples were not analyzed for two days after collection. To test the sampling tubes some air samples were collected in the usual way, and the air analyzed the day the sample was collected, and after standing one, two, and three days. No appreciable alteration in the composition of the air could be detected.

Date.	CO_2	O_2
6–7–26	3·30%	18·11%
7–7–26	3·30%	18·08%
23–6–26	3·50%	16·59%
25–6–26	3·49%	16·61%
25–6–26	3·85%	16·12%
28–6–26	3·83%	16·13%

Three sampling tubes were filled from the same bag at varying stages of passing the contents through the meter. The composition of the air in the three tubes was:

CO_2	3·72%	3·72%	3·71%
O_2	16·86%	16·89%	16·90%

From the above figures it is clear that a good average sample is obtained of the expired air from the Douglas bag, and that no alteration in the composition of the sample collected takes place by leaving it to stand overnight in the sampling tube for analysis.

The determination of the basal metabolic rate was made on 76 subjects, 26 women and 50 men. The subjects were first measured and weighed, and their ages recorded. The standard basal metabolic rates were then calculated according to the Du Bois chart and table, and from the formula of Harris and Benedict.

As can be seen from Table 2, the men showed an average decrease in basal metabolic rate, when compared with the Du Bois predicted value, of 8·9%, with a range of variation of —29·4% to +15·0%. When compared with the Harris and Benedict predicted value the average decrease in basal metabolic rate was 7·6%, with a range of variation of —28·9% to +14·2%. The average respiratory quotient of the men was 0·85.

From Table 1 it can be seen that the basal metabolic rate of the women showed on the average a reduction of 10·5% from the Du Bois predicted value, with a range varying from —33·8% to +11·7%. When compared with the Harris and Benedict predicted value the average decrease in the basal metabolic rate was 8·6%, with a range vary from —31·3% to +10·2%. The average respiratory quotient for the women was 0·83.

Boothy and Sandiford (10) have shown that, by the substitution of certain constants in the Harris and Benedict formulae, surface areas of subjects can be calculated, agreeing remarkably with those calculated by the Du Bois formula. The major discrepancies between the basal metabolic rates as predicted by the Du Bois formulae, and the Harris and Benedict formulae are due to differences in value attributed to factors for age and sex. They conclude that the Du Bois formula for the determination of the surface area and the Du Bois normal standards of heat production for each square metre of body surface for age and sex offer the best means at present available for predicting the normal heat production. The average difference between the two standards in this investigation was 30 calories, while the greatest was 111 calories. In 80% of the subjects the Harris and Benedict value was lower than the Du Bois value.

Comparison of Mean Results with Standards.

The fact that the basal metabolic rates obtained in Sydney were almost all lower than the standard values is interesting, especially since 66% of the results

were outside the range of ±10%, which is usually given as the range for normal individuals, and 23% were outside the range of ±15%, which Barach and Draper (2) and others consider should be allowed as normal variations.

The subjects examined were presumably all normal individuals of good health. All had been in attendance at lectures at the University for over two years, and no abnormal conditions had been observed. One can take it that the results obtained are average normal values for the class of the community represented by the University students, and between the ages of 19 and 25 years.

Twelve of the subjects were in active athletic training; the average variation of their basal metabolic rates was —5·8% from the Du Bois standard, with a range from +15% to —19·9%.

All subjects, except No. 27, were born in Australia.

The three subjects, X, 49, and 50, on whom a number of determinations were made, show much lower average variations from the standards, namely, —19·5%, —22·4%, and —18·0%, from the Du Bois standards, with an average of —20%. These subjects had had great experience in experiments on respiration, and consequently their figures are probably more nearly basal. (See Tables 3, 4, and 5.)

The so-called basal metabolic rate is the measurement under standard conditions of the activity of the vital organs, the tone of the muscles, and is more or less influenced by the emotional state of the subject. Thus a true basal figure cannot be obtained without the full co-operation of the subject, or if the subject is nervous or worried by the procedure. A person repeatedly tested as a laboratory subject will show a lower basal metabolic rate than the same subject would have had in the rôle of a patient.

Unless the basal metabolic rate determinations of a subject agreed within 10% of each other a third determination was made, but it was seen later that a greater variation than this can be allowed. Benedict (4) found a daily variation in a number of individuals to be on an average 13·9%, with a maximum variation of 31·3% and a minimum of 3·5%. Blunt and Dye (8) found a daily variation of their subjects equal to 13·2%, with a maximum of 28·8% and a minimum of 7·4%.

The basal metabolic rate determinations over a period of three years showed a variation of 23·7% for subject X, with 21 determinations, and for subject 50 a variation of 11·2% was obtained with 12 determinations. (See Tables 4 and 5.)

The cause of the day to day variations is not clear, for nothing the subject does or fails to do, except sleep, can lower the heat production, whereas any slight disturbing conditions, such as restlessness, discomfort, etc., may cause an appreciable increase. Temporary elevations in heat production may be due to emotional stimulation of the adrenals, resulting in a discharge into the circulation of an excess of adrenalin, as has been shown by Boothy and Sandiford

(11). The injection of adrenalin is the quickest method known of increasing the metabolism of the body. Thyroxin also increases the metabolism of the body, but it is much slower in action, and the effect lasts for a much longer time.

The Cause of the Lower Basal Metabolic Rate.

The predicted values for the basal metabolic rates of the body have chiefly been worked out in U.S., America, by Du Bois and Harris and Benedict (23), and in England by Dreyer (15), that is, on people accustomed to cool climates. Dreyer's standard metabolic rate figures are on the whole intermediate between those of Du Bois and those of Harris and Benedict.

In Sydney the warmer climate suggests that the lower basal metabolic rate that has been found in this investigation may be due to a climatic effect. The regulation of body temperature within its narrow limits is obtained by a nicely balanced adjustment between heat loss and heat production. Heat loss takes place by purely physical means of conduction, radiation, and evaporation from the surface of the body. Increase or diminution of heat loss is then regulated by the skin circulation and the activity of the sweat glands. Heat production may, however, be altered in two ways: a chemical heat regulation where there is an increase or decrease in the metabolic activity of the tissue cells, such as must take place in fevers or in hyperthyroidism and hypothyroidism; and secondly, a physical heat regulation, where increase or decrease in heat production is brought about by muscular activity.

There has been much controversy on the effect of environmental temperature on the basal metabolic rate, and the greatest amount of attention in the past has been paid to the consideration of the adjustment to a lowered external temperature. The earlier physiologists, e.g., Rubner and Voit, believed in the so-called chemical regulation of body warmth, while more recently the physical mechanism of the regulation of body temperature has been more generally accepted. It is held that the increase in metabolism at low temperatures is due entirely to the muscular activity caused by shivering, goose-skin, or movement, and that, of itself, external cold has no immediate influence on the metabolism of the resting cell. Eykman (18) in 1897, and Ranke (31) in 1900, both found that the energy exchange of the body is the same in summer as in winter if the same amount of work is carried out.

Morgulis (28), who studied the CO_2 production and oxygen absorption of dogs exposed to various temperatures, obtaining at the same time a graphic record of the behaviour of the animals, came to the conclusion that there is no special mechanism of chemical regulation of metabolism; that the metabolic changes produced under lowered temperatures are either the result of shivering and restlessness or lack of relaxation on the part of the subjects. They are due

to differences in muscular tonicity, produced reflexly through cutaneous stimulation. He concludes that the basal metabolic rate may remain unchanged within a temperature range of the environment of 10°C., provided that complete muscular relaxation can be secured.

Rubner (32) found no variation in the metabolism of a dog with alterations in temperature of 20°C. to 30°C., so physical regulation alone sufficed to maintain the temperature of the body at a constant level. At 35°C. there was a decided increase in the heat production, due to a hyperthermia of the cells through insufficient heat loss. Rubner concluded that below 20°C. and above 30°C. a rise in metabolism is obtained, due to chemical regulation, and a minimal metabolism of the body is obtained only when the fasting organism is surrounded by an atmosphere of 30–35°C.

Eykman (19) determined the energy exchanges at rest of Europeans in the tropics and in Europe, and obtained similar figures. His average figures were:

Europeans in Europe	250 cc. of oxygen per minute.
Europeans in East Indies ..	245·7 cc. of oxygen per minute.
Malays in East Indies	251·5 cc. of oxygen per minute.

A study of the food intake of Europeans in Java by Eykman gave an average of 2,400–2,500 calories per day, which is very close to the figure (2,445) obtained by Rubner for inhabitants of Europe.

Other workers, like Glogner (22), Schilling and Jaffe (33), obtained the same results for food consumption of Europeans in the tropics and in Europe, so that it seems that the food intake in the tropics does not differ appreciably from European standards.

From his experiments Eykman (20) came to the conclusion that the metabolism of the body is the same at high as at medium temperatures, and that no adaptation of the organism, subjected to high temperatures, is brought about by a diminution of the energy output of the body.

However, the basal metabolism at high external temperatures is still a matter of discussion. The question whether the body can respond to increases in external temperatures by a reduction in the quantity of heat produced is still undecided. As various workers have found the basal metabolic rates of individuals to be the same in summer as in winter, and also since Eykman (18) found no decrease in the oxygen consumption of white men in the tropics, it has, until very recently, been generally accepted that, under conditions of high external temperatures, the body temperature is regulated purely by physical means.

Breinl and Young (13), from their experiments in Townsville, found in 1920 that the maintenance of body temperature in the tropics is not brought about by any decrease in the heat production of the body. Almeida (1), however, in 1919 determined the basal metabolic rate of 10 white men who were

born and living in tropical Brazil. The result of 26 experiments showed an average basal metabolic rate of 30·35 calories per square metre per hour, with a range from 25·67 to 37·56 calories. Almeida concludes that the inhabitants of warm climates have basal metabolic rates very much lower than those of inhabitants of cold or temperate climates. He points out that this is of great importance from the point of view of acclimatization to warm climates, for it represents the acquisition of a new property, that of reducing the production of heat to a very low level not attained by organisms accustomed to low temperatures.

The results obtained by Rubner, Eykman (32, 19), etc., are attributed to the fact that their subjects had not had time to adapt themselves to the new conditions, which seem to take a long time to become established.

Corlette (14), from theoretical observations, prophesied that the inhabitants of Sydney will be found to have a basal metabolic rate 200 to 500 calories lower than the standard value of Du Bois, which represents the heat loss of an average individual in New York. These results Corlette finds confirmed in the figures for total metabolism in the dietary studies of students in Sydney by Wardlaw (35), who found an average food consumption per day of 1,300 calories for men and 1,283 calories for women. These figures are about 500 calories lower than the Atwater standards for U.S. America.

The basal metabolic rate was found in this investigation to be on an average 150 calories less than the Du Bois value.

Reduced basal metabolic rates have also been found recently in New Orleans. Borgstrom and Hafkesburg (12) found the average basal metabolic rate of 9 trained subjects 18% lower than the Du Bois standard, 16% lower than the Harris and Benedict standard, and 14% lower than the Dreyer standard. Borgstrom does not give any explanation for his low metabolic rates, but suggests that among the factors concerned may be the warm climate and the lower protein intake. He found that protein intake was only 60% of the standard values given for New York.

Similar figures for protein intake have been obtained in Sydney. The results of determinations of blood urea concentration, urea excretion, and protein intake during the past few years in Sydney (Priestley and Hindmarsh, 30), have been much lower than the standard values quoted in text-books, and illustrate the danger of error in taking European and American standards as applicable here.

	Sydney.	America and England.
Blood urea concentration	11 mgm. urea N per 100 cc. blood	12 to 15 mgm.
Urea excretion	27·5 grms. urea in 24 hours ..	30 to 35 grms.
Protein intake	70 grms. in 24 hours	100 grms.

The climate of New Orleans is very similar to that of Sydney, although it is warmer.

From the results obtained in New Orleans, Brazil, and in Sydney, showing a reduction in basal metabolic rate of 18%, 24%, and 20% respectively from the Du Bois standard value, it would seem very probable that acclimatization to high external temperatures does take place, and that the body has the power of reducing its fundamental or basal metabolism to a lower level, thus facilitating the regulation of body temperature where heat loss is difficult.

However, the results of Wishart (37), which have appeared this year, on the basal metabolic rates of subjects in Glasgow, throw fresh light on the hypothesis of a reduction of metabolism as an acclimatization factor in the tropics. The basal metabolic rate of his subjects had been determined a number of times, so that they were trained subjects, and he found the average variation from the Du Bois standard of 3 out of 4 subjects to be —16%, —21·5%, and —21·3% respectively. Pickworth (29) has obtained similar results for the basal metabolic rates of subjects in Birmingham, namely, a reduction of about 20% from the Harris and Benedict predicted values. Pickworth explains his lower figures as due to the more complete muscular relaxation of his subjects than can be obtained by the use of bag methods, since the latter require the use of a mouthpiece or a face mask. Pickworth devised a small airtight chamber, and analyzed the air in it immediately before and a definite time after, usually 1 to 2 hours, the subject had rested quietly within it.

The average variations of the basal metabolic rates from the predicted values of Du Bois, found by the various workers in different parts of the world, and the mean external air temperatures, have been tabulated below.

Place.	Variation of B.M.R. From Du Bois.	Mean temperatures. 3 hottest months.	3 coldest months.	Yearly average.
Rio de Janeiro	—24%	24·2°C.	21·0°C.	22·6°C.
New Orleans	—18%	27·8°C.	13·3°C.	20·7°C.
Sydney (trained subjects)	—20%	21·7°C.	12·2°C.	17·2°C.
New York	—	22·2°C.	0°C.	11·3°C.
Birmingham	—20%	16·0°C.	5°C.	9·6°C.
Glasgow	—20%	11·7°C.	5°C.	9·0°C.

The lower figures for the basal metabolic rates obtained by the various workers mentioned in different parts of the world all show a reduction from the Du Bois standard of the same order. Except in the case of the Rio determinations all the experiments were carried out on trained laboratory subjects. This suggests that the lowered metabolic rates obtained were due to more complete muscular relaxation.

Pickworth claims that complete muscular relaxation cannot be obtained by the bag methods of determining basal metabolic rates, and that it is not possible for a fully trained subject to inhibit muscular tone, voluntary movements, and mental processes when using mouthpieces or face masks. He states, however,

that when a subject is in the chamber for the first time a higher figure for metabolism is noted, which becomes less as the strangeness of the surroundings ceases to attract attention.

Subject 49 (see Table 4), when totally untrained in respiratory experiments, showed a progressive fall in his basal metabolic rate as he became used to the procedure and the apparatus; his results show that muscular relaxation is merely a matter of training and experience, and is not dependent on the type of apparatus used.

In 1876 Zuntz and Pfluger, from their curari experiments, showed that a decrease in muscular tone produced a diminution in the heat formation in muscular tissue, and as a result a fall in the total energy exchange of the body. In 1901 Frank and Voit showed that curari produced no diminution in the metabolism of an animal kept absolutely at rest. Thus the value obtained for the heat production of the body is affected by the state of muscular relaxation at the time of the experiment.

The basal metabolic rate obtained on a subject depends then, after all the standard conditions have been observed, on the degree of muscular relaxation acquired by the individual. In the cooler climates complete muscular relaxation requires practice to attain, but when it has been attained basal metabolic rates at least 20% lower than the Du Bois predicted values are obtained.

The average reduction in basal metabolic rate of 9% from the Du Bois value recorded in this investigation on 73 untrained subjects can then be explained as due to the fact that in the warmer climate of Sydney one relaxes more easily at rest, and in very hot climates, as in Brazil, the reduction of basal heat production by 24%, found by Almeida, points to a still more complete muscular relaxation. The feeling of lassitude in warm, humid climates, and the desire for movement and activity in low external temperatures are indications of the state of muscle tone. In warm-blooded animals body temperature is maintained at a constant level independent of climatic conditions, and the external mechanism for the regulation of body temperature is nervous. The action of cold on the skin stimulates peripheral nerve endings, and reflexly causes a greater heat production by increased tone of muscle, and at the same time reduces heat loss by vasoconstriction of the peripheral vessels. Exposure to heat, on the contrary, causes vasodilatation, and the production of sweat, and a lowering of muscle tone.

Increased metabolism can, of course, be induced without increase of muscular tone, as, for example, in fevers and hyperthyroidism.

The question as to how the organism adapts itself to high external temperatures is of considerable interest, and the lower basal metabolic rate that has been obtained on untrained subjects in warm and hot climates must be regarded as an acclimatization factor. It is suggested that this factor consists in the more ready production and maintenance of muscular relaxation, so that in

climates where heat loss is more difficult there is, during rest, a lessened production of heat. The acquiring of this habit of relaxing readily and without conscious effort takes some time to become established in the body, and is more readily seen in individuals born in warm climates than in those who have migrated there after having adjusted their heat production to a cooler climate.

METHODS OF DETERMINING BASAL METABOLIC RATE.

The methods for the determination of the basal metabolic rate consist either in the measurement of heat loss, direct calorimetry, or in the measurement of heat production, indirect calorimetry. The close agreement between direct and indirect calorimetry has been definitely demonstrated by many workers, e.g., Rubner, Atwater and Benedict, and Du Bois. To measure directly the heat output of the body requires an elaborate and costly apparatus, a calorimeter, and since the essential factors in the formation of body heat are the processes of oxidation, heat production can be determined indirectly by the estimation of the oxygent intake or of the carbon dioxide output. Also Gephardt and Du Bois (21) have shown that because of the many errors in direct calorimetry it is advisable to use the method of indirect calorimetry for short and isolated experiments.

There are three methods in use for the determination of the basal metabolic rate by indirect calorimetry. The first method measures the litres of oxygen absorbed in a definite time, the second the weight of carbon dioxide expired in a definite time, and the third the volume of air expired in a definite time, and requires also the determination of its oxygen and carbon dioxide content.

The first method is the closed circuit or metabolimeter method of Benedict (5). The subject breathes into and out of a spirometer which has been filled with oxygen or air enriched with oxygen; the fall of the spirometer bell registers on a scale the amount of oxygen absorbed or the calorie production per hour. The respiratory quotient is assumed to equal 0·82. The carbon dioxide eliminated is absorbed by passing the expired air over soda-lime before it reaches the spirometer.

This method is very simple to carry out, but it has been greatly criticized by many workers. The main objection to the method seems to be the constant attention required to obtain reliable readings. A leak of only a few cubic centimetres, either in the apparatus or in the adjustment of the mask during the ten-minute determination, will appreciably affect the result, since the leak is measured as a loss of so much oxygen, and is not equivalent to the loss of so much air as in the gasometer method, thus increasing the error five times. The soda-lime also will need frequent changing, and it is difficult to gauge when a portion of the expired carbon-dioxide is escaping absorption. The danger of breathing contaminated air is great, the apparatus needs well sterilizing after each subject, and this is not readily done. This, with the constant checking

required, make it difficult to use the metabolimeter for clinical work where many determinations are to be made. The possible error of this method has been estimated by Meakin and Davies (27) to equal 30%.

The second method is the gravimetric method of King (24). This is an open circuit method; the subject breathes through a mouthpiece or mask which has been fitted with an inlet and an outlet valve. Atmospheric air is inspired, and the expired air is passed through three jars containing calcium chloride, soda-lime, and calcium chloride respectively. The increase in the weight of the second and third bottles is a measure of the expired CO_2 in the time of the experiment. The heat production may be expressed as grammes of CO_2 per square metre per hour, or, using a constant R.Q. of 0·82, the heat production in calories per 24 hours can readily be calculated. This method is very simple to carry out, and does not require very elaborate apparatus. A very accurate balance is necessary, and large supplies of a high-grade soda-lime. King recommends a special soda-lime called Wilson soda-lime, which has greater absorbing powers for CO_2 than the usual soda-lime supplied for chemical work.

Wardlaw (36), in his modification of King's method, states that he uses 600 grms. of Merck's soda-lime: this with the jar will give a total weight of at least 1 kilogramme; the jar of calcium chloride will weigh a like amount. In six minutes, the time of his experiment, about 2 grms. of CO_2 are expired by an adult under basal conditions. This weight is divided between the second and third jars; thus the weighing machine is required to show an increase of perhaps less than 1 grm. in a kilogramme. A balance which can weight 1 kilogramme to 0·1 grm. accurately will give an error of at least 10% in the weighings, whereas if it can weigh 1 kilogramme to 0·01 grm. the error will equal 1%. A large balance with a high degree of accuracy is thus required. The checking of the efficiency of the soda-lime is important. To do this the expired air, after passing through the absorption bottles, is bubbled through lime-water or baryta-water. Wardlaw finds that it is necessary to renew the soda-lime after every ten determinations.

The heat production in calories per 24 hours can be calculated very simply, provided a constant R.Q. of 0·8185 is used instead of 0·82. The error produced by doing this is negligible, and a direct comparison of the heat production of the subject in calories per 24 hours with the predicted values of Du Bois and Harris and Benedict is made very easily. To do this the weight of CO_2 eliminated in 14·4 minutes is determined, and the figure obtained multiplied by 300 gives at once the metabolism of the subject in calories per 24 hours. When the R.Q. equals 0·82 the heat equivalent of 1 grm. of CO_2 is 2·99 calories. When the R.Q. equals 0·8185 the heat equivalent of 1 grm. of CO_2 is 3·00 calories, so that if 5 grms. of CO_2 are eliminated in 14·4 minutes the heat production of the subject is 5×300, or 1,500 calories per 24 hours.

The third method is the gasometer method already described. This is

recognized by most workers as the most accurate method of determining the basal metabolic rate. Its chief drawback is the skill required in the technique of gas analysis.

A Comparison of the Results of the Three Methods.

From the oxygen and CO_2 content of the expired air of the subjects of this investigation, and the volumes of air expired, figures have been calculated to determine what results would have been obtained in each case in basal metabolic rate had the oxygen absorption alone been determined, or the CO_2 excretion alone, such as in the Benedict metabolimeter method or King's gravimetric method.

On the average slightly lower results were obtained with the oxygen absorption method and higher results by the gravimetric method than the gasometer method.

Average Variation from the Du Bois Standard.

Method.	Men.	Women.	Range of Variation. Men.	Women.
Gasometer	—8·9%	—10·5%	+15·0% to —29·4%	+11·7% to —33·0%
O_2 absorption	—9·6%	—10·9%	+15·2% to —30·3%	+9·3% to —33·4%
CO_2 excretion	—6·0%	—7·3%	+15·4% to —25·1%	+21·6% to —35·9%

Range of Variation from the Mean B.M.R. as determined by each method.

Subject.	Gasometer Method.	O_2 Absorption Method.	CO_2 Excretion Method.
47 ..	—4·7% to +5·4%	—5·8% to +6·0%	—9·2% to +5·7%
X ..	—10·0% to +13·0%	—10·0% to +14·0%	—15·5% to +13·9%

These results differ from those of King, who claims that the gravimetric method gives lower figures for basal metabolic rates than either the oxygen absorption method or the gasometer method. He states that "If the basal metabolism is determined by a closed oxygen rich system, twice as many supernormal results may be expected among patients not clinically hyperthyroid as would be obtained by the gasometer method. Among the same group of cases more than twice as many supernormal results may be expected by the Tissot gasometer method as the measurement of CO_2 elimination." It is only fair to point out here that the determinations of basal metabolic rates on which King based the above conclusions were carried out on different groups of subjects and with different sets of apparatus, and one wonders if this is justifiable.

The figures in Tables 6 and 7, calculated for the comparison of the results which would be obtained by the three methods of determining basal metabolic rates by indirect calorimetry, were actually the same experiments, so that they

represent the results on the same group of subjects and with the use of the same sets of apparatus throughout. This seems a fairer comparison.

The percentage variations of the abnormal results obtained by the three methods in this investigation are tabulated below. A variation of ±10% was taken as the normal range. The variation was calculated from the average variation of all the subjects from the Du Bois standard obtained by each method. Thus in the gasometer method the normal range was considered to be ±10% from a standard 8·9% below Du Bois for men and 10·5% below the Du Bois standard for women.

Method.	% Abnormal Results.	% Supernormal Results.	% Hyponormal Results.
Gasometer	18%	11%	7%
O_2 absorption ..	18%	11%	7%
CO_2 excretion ..	19%	11%	8%

These results show that the same number of abnormal results should be obtained in the basal metabolic rate determinations by all three methods. Differences then that are obtained must be due to errors in technique of the method, and if lower results are obtained by the gravimetric method, then the whole of the CO_2 expired has not been taken up by the soda-lime.

It became evident while carrying out this investigation that often the first basal metabolic rate determination was higher than the succeeding ones, and that this was associated with a higher volume output of air than normal and a high R.Q. The CO_2 excretion method in these cases gave much higher results than the O_2 absorption methods. (These results are marked with an asterisk in Tables 1 and 2.)

King claims that the analysis of 157 published observations on gas exchange showed that CO_2 is either not washed out during the practical application of the basal metabolic rate test, or else it is washed out in negligible amounts.

Boothy and Sandiford (9) point out that it is important to impress upon patients the necessity of breathing naturally. The tendency of patients to vary the depth and regularity of their respirations during the tests does not affect the total metabolism materially, but it does affect the R.Q. Abnormal R.Q.'s above 1·00 or below 0·7 indicate as a rule a nervous condition of the patient or an error in technique. They state that it is advisable to repeat such cases, although they have rarely found a sufficient change in the check determination to affect the clinical value of the report. In this investigation it was found that irregular respiration, affecting as it does the lung ventilation of the subject, may affect the metabolism figure obtained appreciably.

Some experiments were then carried out to study the effect of increasing lung ventilation. The increase in the rate of respiration and the increase in the depth were taken separately. The results are given in Tables 8 and 9, and

it is very evident from them that increased lung ventilation causes a marked rise in the metabolism figure obtained in the R.Q. The latter is due to the fact that increased lung ventilation washes out CO_2 at a greater rate than it increases the intake of oxygen.

Table 11 gives a summary of the results of these experiments, and shows that increased lung ventilation by increased depth affects the basal metabolic rate determination to a greater extent than increased rate, and by both processes the metabolism figure obtained is markedly increased.

The increased elimination of CO_2 obtained by increased rate of respiration does not agree with the results of Lossen and quoted by Lusk. Lossen found that the CO_2 elimination was independent of the ventilation of the lungs.

When the basal metabolism rate determination is calculated from the CO_2 elimination alone the increase in the figure obtained after increasing lung ventilation is much greater than when calculated on the oxygen intake alone or by using the oxygen intake and the variable R.Q.

King (25) claims that the CO_2 output is less variable than the oxygen intake, and that $C0_2$ elimination is possibly a more accurate index to heat production than is oxygen consumption. The experiments on which these conclusions are based were continued for a period of one hour, and this is not possible with patients in general for indirect calorimetry. Errors would at once appear, due to restlessness. In short period experiments, 10 to 15 minutes' duration, a subject is much more likely to increase the output of CO_2 than in an experiment of one hour, for in the latter there would be time to compensate for the early washing out of CO_2 by a later retention.

When the R.Q. equals 0·82 no difference is obtained in the basal metabolic rate, whether determined by the oxygen absorbed, CO_2 output, or the gasometer method, but in subjects who show an appreciable variation in the volume output of expired air the basal metabolic rates recorded by the CO_2 method are much higher than those determined by the oxygen absorption or the gasometer methods.

Normal subjects, in the strict basal conditions required for a basal metabolic rate determination, have an R.Q. of 0·82, and any increase in this indicates a high basal metabolic rate determination, due to increased lung ventilation or to nervous stimulation. This suggests then that a basal metabolic rate determination which has an R.Q. of over 0·9 should be repeated, and perhaps discarded. The range of variation for normal metabolic rates is such that small increases in the lung ventilation or slight nervous stimulation are not of importance clinically. Occasionally one finds a subject who will over-breathe, even when the test has been repeated three times, and it is well to realize that as a basal metabolic rate determination the results are useless. Subject (a), Table 1, is an example of this.

The experiments which recorded R.Q.'s approaching 1·00, and where the volume of air expired was appreciably greater than the other tests on the same

subject, were not used in calculating the average basal metabolic rate of the subjects in Tables 1 and 2.

A high R.Q. is not always associated with a high volume output of expired air, but where it was it seemed advisable to discard the results.

In determining the basal metabolic rate then by the gasometer method one has the advantage of checking the test in this way, for an R.Q. of over 0·9 arouses suspicion of faulty technique or the collection of an abnormal sample. The results obtained on subject 49 are of interest here. The basal metabolic rate was determined for 5 consecutive days; the first three days recorded a consistent fall in the volume of expired air, the R.Q., and the basal metabolic rate. In the first experiment the basal metabolic rate determined from the CO_2 excretion gave a figure 140 calories higher than the gasometer method, and 170 calories higher than the oxygen absorption method, but in the later experiments, when a sample of air more nearly basal was collected, there were no appreciable differences in the figures obtained by the three methods.

Except for gross manifestations like movement, excitement, etc., there is no method of gauging whether a true basal metabolic rate is being determined or not, since one cannot estimate the degree of muscular relaxation and the psychological condition of the subject.

One realizes that a high basal metabolic rate in normal subjects may be due not only to increased lung ventilation, the nervous factor is a large and important one in a number of cases, but the fact that increases in lung ventilation can so easily be obtained, and cause a high figure to be given for basal metabolic rate, is important, especially since breathing through a mask or mouthpiece tends of itself to increase lung ventilation.

The normal range of variation of the volume output of expired air from 21 experiments on subject X was —19% to +10% from the average, and for 12 experiments on subject 50 from —18% to +14%. Thus a normal range of variation in lung ventilation was found to be about 30% in trained subjects.

Moderate increases in the lung ventilation may show appreciable increases in the basal metabolic rate.

Increase in Lung Ventilation.	Increase in B.M.R. Gasometer Method.	Increase in B.M.R. CO_2 Excretion Method.	Effect on R.Q.
32%	7%	10%	0·85 to 0·85
39%	9%	—9%	0·85 to 0·67
48%	22%	36%	0·84 to 0·97
52%	14%	31%	0·82 to 0·99
56%	19%	43%	0·90 to 1·11

The contention that in a 15-minute collection of expired air the increased lung ventilation will only continue for the first 5 minutes, and that during the

next 10 minutes any washing out of CO_2 will be compensated by a retention of CO_2 is not borne out by the results of some experiments on voluntarily increasing lung ventilation.

The results in Table 12 show that in an experiment where increased lung ventilation was maintained for 15 minutes, and the samples of air were collected at 5-minute intervals, more CO_2 was eliminated in the last 5 minutes than in the first or second samples. The subject did not find it difficult to maintain this increased breathing, nor suffer from any after-effects. In the other similar experiments (Tables. 11, 13, and 14), although the CO_2 excretion fell steadily in the three 5-minute samples of air, the third sample contained over 50% more CO_2 than normal for the subject. These experiments show very exaggerated conditions of superventilation, still one sometimes collects 100 litres of air from a subject, and it is well to recognize that these increased lung ventilations can be maintained quite easily. If the subject is inclined to over-breathe, it is better to discard the figures and repeat the tests at a future date.

If over-ventilation of the lungs takes place to any extent a normal sample of expired air cannot be obtained for at least half an hour afterwards, for it takes some time for the alveolar CO_2 to reach its normal level again. (Table 8.)

With subject X the normal basal metabolic rate was 1,158 calories per 24 hours. On increasing lung ventilation from 50·8 litres to 156 litres in 14·4 minutes the metabolic rate obtained was 1,494 calories; a quarter of an hour later the normally expired air sample gave a metabolism figure of 866 calories per 24 hours, with a volume output of air of only 36·8 litres.

A determination of the composition of alveolar air, obtained by collecting the last portion of a deep expiration, was carried out. No alteration in the alveolar CO_2 and oxygen content was obtained before and after giving a sample of expired air for 14·4 minutes into a Douglas bag. It was found that after forced breathing the alveolar oxygen had increased from 14·42% to 17·19%, and the alveolar CO_2 had decreased from 5·47% to 3·66%. This means that at the end of 14·4 minutes increased lung ventilation there was present in the body (mostly in the lungs, and to a lesser extent in the blood) an extra 122 cc. oxygen. This is measured as oxygen absorption, so an increase in the metabolism figure is obtained. Similarly the washing out of 1,430 cc. of extra CO_2 is measured as CO_2 production by the body during the experiment.

The collection of the last portion of a deep expiration after holding the breath for 30 seconds was next carried out, as it was hoped by this means to obtain some idea of the amount of oxygen and CO_2 in the venous blood after normal respirations and after superventilation of the lungs. The results are given in Tables 11, 13, and 14, but the figures obtained were not constant enough to enable one to calculate the actual amount of increase in the venous oxygen tension or the lowering of the CO_2 venous tension.

The CO_2 output and the oxygen intake as determined by the Douglas bag sample, may not be a true index of the CO_2 produced and of the oxygen absorbed by the body in the time of the test. The normality of the sample of expired air collected may be tested by a determination of the alveolar CO_2 and oxygen of the subject immediately before the collection of the Douglas bag sample, and again immediately after the sample has been collected. If the alveolar oxygen percentage is higher, and the CO_2 percentage is lower in the second sample of alveolar air, then it is obvious that the subject has over-ventilated the lungs during the test.

In the gasometer method of determining the basal metabolic rate one has a check on the normality of the sample collected from the volume of the expired air and the R.Q. If there is still some doubt then, in subsequent estimations, the determination of the oxygen and CO_2 content of the alveolar air of the subject immediately before and immediately after the collection of the sample gives definite information as to whether over-ventilation has or has not taken place.

In conclusion, I wish to express my thanks to Professor H. G. Chapman, in whose laboratory this work was carried out, for his advice, and also to Associate Professor Priestley for his helpful criticism.

SUMMARY.

1. The basal metabolic rate of 76 subjects was determined by the gasometer method. An average reduction of 8·9% was obtained for the men and 10·5% for the women when compared with the Du Bois predicated values.

2. It is suggested that the reduced basal metabolic rate is due to the more ready muscular relaxation of the subjects in the warmer climate of Sydney.

3. Basal metabolic rate determinations from oxygen absorption alone, using a fixed R.Q., showed an average reduction of 9·6% for men and 10·9% for women when compared with Du Bois predicted values.

4. Basal metabolic determinations from CO_2 output alone, with a fixed R.Q., showed an average reduction of 6·0% for men and 7·3% for women.

5. The gasometric method, where the oxygen absorbed, the CO_2 excreted, and the respiratory quotient are all determined, should give the most consistent results for basal metabolic rate determinations.

6. The determination of R.Q. and volume output of expired air is a useful check on the normal breathing of a subject.

7. Determinations of alveolar CO_2 and oxygen immediately before and immediately after the test give the most accurate information of the normality of the sample collected.

TABLE 1. Women.

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour
A	19	1·52	67	98·3°F.	—	71·0	3·55	0·932	1252	1268	1386	1341	—8·4	—5·4	34·8
			70	98·5°F.	—	69·1	3·99	0·779	1284						
B	19	1.55	65·68	98·4°F.	18	79·3	3·68	0·827	1408	1309	1414	1370	—7·4	—4·5	35·2
			65·65	98·6°F.	18	61·4	4·01	0·773	1175						
			62·64	98·0°F.	—	66·9	4·11	0·783	1313						
			60	98·0°F.	14	69·0	4·03	0.811	1338						
C	19	1·72	—	—	—	68·8	4·11	0·849	1374	1288	1569	1485	—18·5	—13·9	31·0
			73	—	—	76·6	3·26	1·01	1260*						
			—	—	—	65·0	3·87	0·778	1202						
D	20	1·42	—	—	—	88·5	3·15	0·914	1381	1428	1278	1296	+11·7	+10·2	41·9
			—	—	—	79·8	3·80	0·871	1478						
			69	—	—	79·0	3·64	0·923	1424						
E	20	1·43	78	98·2°F.	—	73·6	3·38	0·985	1251*	1	1287	1302	—12·6	—13·5	34·0
			74	98·7°F.	14	61·4	3·65	0·871	1095	1126					
			—	98·5°F.	—	55·0	4·34	0·838	1158						
F	20	1·45	74	98·2°F.	—	48·7	4·63	0·931	1120	1200	1305	1317	—8·0	—8·9	34·5
			72	98·3°F.	12	56·8	4·61	0·874	1281						
G	20	1·48	60	98·2°F.	—	62·7	3·70	0·870	1135	1169	1332	1344	—12·2	—13·0	32·9
			60	98·1°F.	12	55·2	4·51	0·829	1204						
H	20	1·59	—	98·8°F.	16	66·6	3·63	0·928	1198	1227	1431	1375	—14·3	—10·8	32·2
			—	—	—	63·6	3·99	0·922	1256						
I	20	1·61	60·62	98·4°F.	18	74·5	3·94	0·817	1419	1305	1448	1451	—9·9	—10·1	33·8
			60·62	98·0°F.	18	72·1	3·51	0·897	1246						
			60·60	98·5°F.	18	70·3	3·71	0·787	1249						

TABLE 1. Women (continued).

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour
J	21	1·42	—	—	—	80·1	3·48	0·744	1320*	—	1261	1274			
			—	—	—	99·7	2·37	0·962	1181*						
			—	—	—	93·0	2·18	1·01	1023*						
K	21	1·50	—	—	—	54·4	4·34	0·829	1141	1128	1331	1337	—15·2	—15·6	31·3
			—	—	—	57·8	3·94	0·883	1116						
L	21	1·54	76	98·2°F.	—	69·1	3·49	1·03	1217	1220	1367	1347	—10·8	—9·4	33·0
			80	98·8°F.	—	68·4	3·63	0·900	1222						
M	21	1·57	69·69	98·6°F.	17	63·3	4·59	0·756	1377	1336	1394	1367	—4·2	—2·3	35·5
			68·67	98·6°F.	12	61·6	4·19	0·753	1294						
N	21	1·59	—	98·0°F.	—	62·2	3·44	0·816	1031	1238	1411	1364	—12·3	—9·2	32·5
			70	98·4°F.	—	76·3	3·83	0·812	1407						
			—	—	—	74·4	3·51	0·866	1276						
O	21	1·76	54	—	—	55·5	5·29	0·796	1409	1396	1562	1516	—10·6	—7·9	33·1
			—	—	—	59·3	4·84	0·812	1382						
P	21	1·65	64	98·2°F.	—	63·7	4·60	0·809	1409	1215	1465	1433	—17·1	—15·2	30·7
			—	—	—	63·8	3·76	0·849	1167						
			75	—	—	51·5	4·28	0·836	1069						
Q	22	1·62	79·73	98·6°F.	17	65·7	4·22	0·805	1333	1335	1438	1400	—7·2	—4·6	34·3
			70·78	98·3°F.	18	70·7	3·98	0·759	1337						
R	22	1·76	60·58	98·7°F.	20	76·2	3·70	0·770	1344	1353	1563	1490	—13·4	—9·2	32·0
			59·64	98·4°F.	17	74·0	3·83	0·804	1362						
S	23	1·52	66·67	98·6°F.	14	65·3	3·92	0·824	1236	1255	1350	1335	—7·0	—6·0	34·4
			60	—	—	69·6	3·58	0·981	1258						
			69·70	98·0°F.	16	63·1	4·11	0·883	1272						

TABLE 1. Women (continued).

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour per hour
T	24	1·80	—	—	—	63·4	3·50	0·857	1081	1056	1598	1538	—33·8	—31·3	24·5
			60	97·1°F.	20	64·6	3·13	0·817	975						
			56	97·2°F.	—	64·1	3·65	0·671	1112						
U	25	1·40	70	98·8°F.	—	48·1	4·97	0·791	1145	1145	1243	1255	—7·9	—8·8	34·1
V	26	1·62	84	98·0°F.	12	61·7	5·08	0·752	1487	1393	1438	1425	—3·1	—2·2	35·8
			78·79	98·0°F.	12	65·4	4·75	0·760	1476						
			67·69	98·1°F.	12	59·1	4·45	0·881	1291						
			67·67	98·4°F.	12	62·6	4·37	0·821	1319						
W	40	1·39	68·70	98·7°F.	12	52·2	4·23	0·825	1066	1066	1209	1162	—11·8	—8·3	32·0
X	32	1·46	—	—	—	49·8	4·36	0·824	—	1046	1297	1238	—19·5	—15·5	29·8
Y	40	1·43	72·73	98·0°F.	6	46·7	5·19	0·815	1170	1149	1244	1176	—7·6	—2·3	33·5
			70·70	98·0°F.	6	49·4	4·73	0·818	1128						
Z	56	1·68	—	—	—	69·7	4·13	0·871	1407	1407	1411	1359	—0·3	+3·5	34·9
a	12	1·52	73	99·1°F.	22 23 26	97·1	3·32	0·904	1589	1589	1641	—	—3·2	—	43·5
			64	98·9°F.	26	168·0	2·12	1·29	1797*						
			—	98·9°F.	20	158·6	2·37	1·28	1896*						
								Averages	0·834				—10·5	—8·6	
								Standard Deviations ..					22·1	18·7	
								Probable Errors of the Averages					±2·92	±2·52	

*Abnormal results not considered in averages. See text.

TABLE 2. Men.

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M.
1	19	1·61	—	—	12	68·8	4·89	0·818	1623	1675	1584	1524	+6·2	+9·9	43·4
			—	—	9	56·9	6·33	0·687	1677						
			—	98·6°F.	6	62·6	5·81	0·762	1725						
2	19	1·62	70	98·4°F.	—	53·3	4·33	0·965	1155	1155	1594	1545	—27·5	—24·6	29·7
			60	98·4°F.	—	74·0	3·56	0·991	1326*						
			52	98·8°F.	—	65·6	3·30	1·02	1091*						
3	19	1·77	—	—	14	72·3	4·27	0·866	1508	1510	1742	1678	—13·3	—10·0	35·6
			—	—	12	68·5	4·64	0·827	1536						
			—	98·2°F.	11	70·0	4·33	0·879	1486						
4	19	1·78	—	—	—	69·9	4·53	0·984	1592	1605	1751	1708	—8·3	—6·0	37·5
			—	—	—	76·6	4·33	0·889	1630						
			—	—	—	61·3	5·44	0·783	1594						
5	19	1·79	—	—	—	71·0	4·78	0·790	1624	1577	1761	1680	—10·5	—6·1	36·7
			—	98·4°F.	14	68·0	4·91	0·798	1601						
			—	98·4°F.	16	72·8	4·27	0·838	1507						
6	19	1·94	64	97·8°F.	14	77·5	4·29	0·843	1613	1613	1909	1849	—14·4	—12·8	34·6
7	19	1·94	—	—	—	83·4	4·52	0·973	1886	1977	1824	1839	+8·4	+7·5	42·4
			—	—	—	83·6	4·99	0·930	2069						
8	19	1·68	67	98·8°F.	—	61·6	3·97	0·826	1181	1182	1653	1585	—28·4	—25·4	29·3
			66	98·8°F.	—	69·4	3·47	0·890	1183						
9	20	1·65	—	—	—	78·9	4·00	0·872	1543	1532	1584	1575	—3·3	—2·7	38·7
			—	—	—	68·9	4·64	0·769	1522						
10	20	1·67	—	—	—	77·7	3·55	0·927	1366	1426	1603	1569	—11·0	—9·1	35·3
			—	—	—	84·9	3·60	1·06	1546*						
			—	—	—	71·4	4·25	0·875	1486						

TABLE 2. Men (continued).

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour
11	20	1·68	—	—	10	52·0	4·82	0·912	1236	1322	1612	1581	—18·0	—16·4	32·8
			—	—	9	51·5	5·19	0·807	1286						
			—	—	8	57·8	5·15	0·844	1444						
12	20	1·68	—	—	11	66·2	4·20	0·885	1364	1418	1613	1585	—12·1	—10·5	35·2
			—	98·0°F.	14	71·4	4·25	0·856	1471						
13	20	1·69	—	—	—	69·7	3·88	0·951	1347	1442	1622	1588	—11·1	—9·2	35·6
			—	—	—	69·5	4·53	0·905	1553						
			—	—	—	62·9	4·58	0·921	1425						
14	20	1·71	72·69	98·2°F.	—	91·2	3·39	0·908	1526*	—	1642	1618	—12·6	—11·4	34·9
			66	98·4°F.	17	109·2	2·71	1·19	1499*						
			68·67	98·0°F.	—	67·3	4·43	0·821	1433	1433					
15	20	1·75	—	—	—	64·4	4·33	0·888	1370	1393	1680	1676	—17·1	—16·8	33·2
			—	—	—	64·4	4·49	0·875	1416						
16	20	1·75	—	—	—	85·9	3·79	0·881	1594	1497	1680	1679	—10·9	—10·8	35·7
			—	—	—	83·9	3·60	0·927	1496						
			—	—	—	68·9	4·20	0·828	1402						
17	20	1·75	—	—	11	63·2	4·93	0·850	1515	1432	1680	1637	—14·8	—12·5	34·1
			—	—	12	60·8	4·44	0·952	1348						
18	20	1·76	—	—	12	70·5	4·33	0·794	1464	1541	1690	1665	—8·8	—7·4	36·5
			—	—	12	77·6	4·32	0·826	1619						
			—	—	15	81·4	3·88	0·866	1540						
19	20	1·78	—	—	10	69·1	4·91	0·876	1661	1678	1709	1689	—1·8	—0·7	39·3
			—	—	12	70·0	4·94	0·902	1704						
			76·64	98·3°F.	12	68·4	5·04	0·837	1670						
20	20	1·79	—	—	14	78·7	4·55	0·835	1734	1658	1719	1718	—3·5	—3·5	38·6
			—	—	17	75·8	4·45	0·840	1635						
			—	—	—	72·8	4·57	0·822	1606						

TABLE 2. Men (continued).

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour
21	20	1·82	—	98·6°F.	11	66·6	4·63	0·861	1504	1523	1747	1726	—12·8	—11·8	34·9
			—	98·4°F.	10	64·2	4·90	0·876	1541						
22	20	1·87	70	—	—	72·1	4·05	0·911	1442	1537	1795	1804	—14·4	—14·8	34·1
			64	94·4°F.	—	81·9	4·08	0·863	1631						
23	20	1·96	65	—	—	65·0	5·04	0·795	1571	1507	1882	1868	—19·9	—19·4	32·0
			60	—	—	67·7	4·43	0·817	1442						
			—	—	—	78·4	3·89	0·913	1507						
24	20	1·90	—	—	—	80·6	5·02	0·836	1956	1848	1824	1839	+1·3	+0·5	40·5
			—	—	—	74·4	4·79	0·866	1740						
			—	—	—	107·6	3·89	1·01	2113*						
25	21	1·62	—	—	15	79·9	3·64	0·975	1460	1493	1536	1507	—2·8	—0·9	38·4
			—	98·9°F.	19	84·1	3·68	0·708	1525						
26	21	1·67	70·70	98·2°F.	11	66·2	5·13	0·850	1652	1649	1583	1574	+4·2	+4·7	41·2
			69·68	98·2°F.	10	66·3	5·00	0·836	1606						
			69·70	98·3°F.	12	68·9	4·90	0·844	1640						
27	21	1·72	—	—	—	71·1	4·32	0·896	1511	1511	1630	1617	—7·3	—6·5	36·6
			—	—	—	82·4	3·99	0·990	1655*						
			—	—	—	89·2	3·96	0·944	1759*						
28	21	1·72	—	98·6°F.	8	72·6	4·55	0·824	1596	1619	1630	1647	—0·7	—1·7	39·2
			—	98·3°F.	14	72·2	4·75	0·884	1682						
			—	98·7°F.	8	66·2	4·88	0·869	1579						
29	21	1·73	—	—	18	73·3	3·89	0·897	1401	1428	1640	1622	—12·9	—12·0	34·4
			80	98·7°F.	16	68·4	4·64	0·756	1508						
			82·82	98·6°F.	15	70·0	3·99	0·899	1375						
30	21	1·74	—	—	—	80·6	3·82	1·01	1554*	1561	1649	1628	—5·5	—4·3	37·3
			—	—	—	72·1	4·35	0·944	1561						

TABLE 2. Men (continued).

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour
31	21	1·78	78	—	—	80·2	4·51	0·851	1758	1804	1687	1670	+6·9	+8·0	42·2
			78·76	99·0°F.	12	78·5	4·60	0·865	1763						
			78	99·0°F.	12	81·0	4·82	0·836	1892						
32	21	1·79	—	98·2°F.	14	76·8	4·41	0·789	1622	1543	1697	1686	—9·1	—8·5	35·9
			—	98·4°F.	9	60·1	5·23	0·835	1521						
			68	98·6°F.	—	61·8	4·86	0·913	1485						
33	21	1·77	—	—	10	59·2	5·10	0·845	1467	1415	1678	1688	—15·7	—16·2	33·3
			—	—	9	51·2	5·59	0·772	1362						
34	21	1·93	—	98·5°F.	10	95·7	3·70	1·07	1787*	—	1830	1822			
			—	98·5°F.	7	71·5	5·11	0·812	1759	1684	—	—	—8·0	—7·5	36·4
			—	98·4°F.	8	69·1	4·75	0·884	1609						
35	21	1·70	—	—	—	88·8	4·01	1·05	1797*	1632	1611	1590	+1·3	+2·4	40·0
			—	—	—	74·7	4·97	0·913	1834*						
			—	—	—	72·9	4·50	0·940	1632						
36	21	1·96	—	—	—	81·3	5·07	0·879	2020	1982	1858	1891	+6·7	+4·8	42·1
			—	—	8	78·9	4·95	0·854	1901						
			—	—	12	80·5	5·15	0·864	2024						
37	21	2·04	74	—	—	76·2	43·0	1·01	1652*						
			68	—	—	66·3	52·3	0·871	1695	1695	1934	1987	—12·4	—14·7	34·6
38	22	1·74	—	—	—	63·0	4·56	0·820	1385	1364	1649	1625	—17·3	—16·1	32·7
			—	98·4°F.	12	64·8	4·82	0·784	1493						
			—	—	—	59·0	4·16	0·911	1213						
39	22	1·85	74	98·2°F.	10	63·6	5·13	0·760	1550	1536	1754	1733	—12·4	—11·4	34·6
			74	97·9°F.	14	68·6	4·89	0·730	1584						
			72	98·5°F.	—	71·6	4·29	0·800	1475						
40	22	1·85	—	98·0°F.	9	64·8	4·64	0·808	1447	1498	1754	1745	—14·6	—14·2	33·8
			—	98·1°F.	8	72·1	4·46	0·820	1550						

TABLE 2. Men (continued).

TABLE 2. Men (continued).

Subject	Age Years	Surface Area Sq. M.	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in Litres 14·4 Minutes	% Oxygen Absorbed	R.Q.	Calories per 24 Hours. Basal Metabolism Gasometer Method	Average B.M.	Du Bois Standard	Harris and Benedict Standard	% Variation of Average from Du Bois	% Variation of Average from Harris and Benedict	Calories per Sq. M. per hour
41	22	1·93	—	—	10	78·2	5·50	0·805	2065	2105	1830	1843	+15·0	+14·2	45·4
			—	—	10	77·6	5·76	0·779	2134						
			—	—	10	80·9	5·37	0·862	2115						
42	22	1·65	—	—	—	71·3	4·76	0·842	1646	1596	1564	1539	+2·0	+3·7	40·3
			65	—	—	75·9	4·26	0·782	1545						
43	23	1·63	—	97·9°F.	12	67·2	4·13	0·934	1378	1320	1545	1516	—14·5	—12·9	33·8
			72	98·1°F.	12	67·6	3·94	0·934	1322						
			69	—	—	54·0	5·20	0·742	1327						
			—	—	—	63·3	4·01	0·902	1251						
44	23	1·65	—	98·1°F.	12	68·7	4·64	0·763	1516	1512	1564	1554	—3·3	—2·7	38·2
			—	98·5°F.	11	69·5	4·53	0·794	1508						
45	25	1·63	73	98·6°F.	16	70·0	4·64	0·836	1574	1587	1545	1504	+2·7	+5·5	40·6
			—	98·0°F.	—	82·2	3·96	0·894	1600						
46	25	1·86	60	97·9°F.	—	74·3	3·38	1·04	1267*						
			56	98·0°F.	—	63·7	4·00	0·872	1245	1245	1763	1752	—29·4	—28·9	27·8
47	26	1·55	—	—	—	75·6	3·71	0·911	1385	1476	1469	1425	+0·2	+3·6	39·7
			—	—	—	79·0	3·96	0·856	1524						
			—	—	—	79·5	4·04	0·829	1518						
48	27	1·85	—	98·1°F.	8	71·0	4·87	0·903	1703	1746	1754	1713	—0·1	+2·1	39·3
			—	98·2°F.	7	59·4	6·28	0·794	1789						
49	27	1·63	—	—	—	50·7	4·97	0·788	—	1198	1545	1468	—22·4	—18·4	30·5
50	42	1·85	—	—	—	75·7	3·84	0·836	—	1408	1710	1599	—18·0	—12·3	31·7
				Averages				0·850					—8·9	—7·6	
				Standard Deviations ..									19·9	17·6	
				Probable Errors of the Averages									±1·89	±1·68	

*Abnormal results not considered in averages. See text.

TABLE 3. Subject X.

Date	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in 14·4 Minutes	% Oxygen Absorbed	% CO_2 Expired	R.Q.	Basal Metabolism: Gasometer Method	Basal Metabolism: O_2 Method	Basal Metabolism: CO_2 Method	Du Bois Standard	Harris and Benedict Standard
7-25	—	—	—	49·8	4·20	3·72	0·878	1024	1009	1091	1297	1238
7-25	—	—	—	50·0	4·31	3·24	0·745	1021	1040	956		
7-25	—	—	—	50·7	4·07	3·33	0·811	994	996	996		
7-25	—	—	—	54·3	4·23	3·35	0·784	1098	1108	1072		
7-25	—	—	—	48·2	4·30	3·15	0·726	976	1000	895		
8-25	—	—	—	53·3	3·81	3·34	0·869	992	980	1049		
8-25	—	—	—	54·8	3·83	3·35	0·867	1025	1013	1081		
23- 9-25	80	—	—	53·0	3·81	3·34	0·869	987	974	1043		
30- 9-25	78	—	—	50·3	3·83	3·35	0·867	940	930	993		
7-10-25	78	—	—	51·3	4·60	3·91	0·843	1146	1138	1182		
15-12-25	—	—	11	40·2	5·09	3·93	0·766	974	987	931		
2-26	78	97·5°F.	12	53·2	4·34	3·58	0·818	1112	1114	1122		
16- 6-26	80	98·6°F.	10	49·0	4·63	3·78	0·809	1092	1095	1092		
18-11-26	77	98·7°F.	10	50·8	4·77	3·75	0·779	1158	1167	1120		
25-11-26	69	98·5°F.	10	46·0	4·56	3·90	0·848	1020	1015	1061		
30-11-26	69	98·6°F.	9	44·7	4·78	4·05	0·841	1036	1031	1067		
1-12-26	74	99·0°F.	10	51·4	4·80	3·94	0·814	1189	1190	1194		
5- 2-27	72	98·0°F.	10	48·8	4·40	3·98	0·900	1057	1036	1145		
7- 2-27	70	97·7°F.	10	50·2	4·22	3·59	0·844	1031	1022	1062		
2- 3-27	68	—	9	47·6	4·44	3·59	0·801	1017	1020	1009		
9- 3-27	71	—	9	47·8	4·67	3·87	0·822	1075	1077	1088		
Average			.. ..	49·8	4·36	3·62	0·824	1046	1045	1059		

TABLE 4. Subject 49.

Date	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in 14·4 Minutes	% Oxygen Absorbed	% CO_2 Expired	R.Q.	Basal Metabolism: Gasometer Method	Basal Metabolism: O_2 Method	Basal Metabolism: CO_2 Method	Du Bois Standard	Harris and Benedict Standard
9-5-27	—	—	20	83·9	3·66	3·35	0·907	1515	1482	1657	1545	1468
10-5-27	65	—	16	60·0	4·49	3·67	0·811	1298	1300	1298		
11-5-27	60	98·7°F.	11	50·9	5·04	3·98	0·784	1226	1238	1197		
12-5-27	58	98·6°F.	9	45·9	5·38	4·24	0·783	1180	1191	1147		
13-5-27	59	98·2°F.	14	55·2	4·49	3·61	0·797	1188	1196	1174		
	Average	..	. . .	50·7	4·97	3·94	0·788	1198	1208	1173		

TABLE 5. Subject 50.

Date	Pulse	Mouth Temperature	Respiration Rate	Volume Expired in 14·4 Minutes	% Oxygen Absorbed	% CO_2 Expired	R.Q.	Basal Metabolism: Gasometer Method	Basal Metabolism: O_2 Method	Basal Metabolism: CO_2 Method	Du Bois Standard	Harris and Benedict Standard
7-25	—	—	—	78·7	3·63	3·10	0·846	1386	1378	1437	1710	1599
7-25	—	—	—	62·4	4·61	3·55	0·763	1367	1388	1306		
8-25	—	—	—	77·8	3·87	3·18	0·814	1451	1453	1458		
8-25	76	—	—	76·7	3·78	2·97	0·777	1383	1399	1343		
25- 2-26	—	—	—	76·8	3·55	3·32	0·926	1352	1316	1503		
4- 3-26	—	—	—	72·8	3·77	3·32	0·873	1342	1324	1416		
23-11-26	67	98·4°F.	12	71·6	4·02	3·43	0·846	1399	1390	1451		
26-11-26	67	98·0°F.	10	78·2	3·66	3·26	0·882	1403	1383	1503		
2-12-26	58	98·0°F.	10	76·9	3·72	3·34	0·889	1404	1383	1511		
7- 2-27	66	98·0°F.	12	70·9	4·16	3·31	0·788	1411	1423	1383		
3- 3-27	—	—	12	79·1	3·88	3·26	0·832	1485	1481	1521		
8- 3-27	53	—	15½	86·4	3·48	2·83	0·805	1448	1451	1430		
	Average	..	..	75·7	3·84	3·24	0·836	1408	1397	1439		

TABLE 6. Women.

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
A	1252	1217	1398			
	1284	1297	1246	—8·4	—9·3	—4·6
	1268	1257	1323			
B	1408	1407	1434			
	1175	1189	1134			
	1313	1326	1281	—7·4	—6·9	—8·2
	1338	1341	1341			
	1309	1316	1298			
C	1374	1363	1426			
	1260	1204	1508	—18·5	—19·7	—12·8
	1202	1214	1167			
	1279	1260	1367			
D	1381	1345	1522			
	1478	1458	1561	+11·7	+9·3	+21·6
	1424	1387	1582			
	1428	1397	1554			
E	1095	1080	1161			
	1158	1152	1191	—12·6	—13·3	—8·6
	1126	1116	1176			
F	1120	1089	1248			
	1281	1264	1353	—8·0	—9·9	—0·5
	1200	1176	1299			
G	1135	1120	1203			
	1204	1201	1227	—12·2	—12·8	—8·8
	1169	1161	1215			
H	1198	1164	1333			
	1256	1224	1392	—14·3	—16·5	—4·8
	1227	1194	1362			
I	1419	1420	1537			
	1246	1221	1352	—9·9	—10·2	—5·3
	1249	1259	1223			
	1305	1300	1371			
J	1320	1345	1237			
	1181	1139	1357	—6·8	—8·5	+0·9
	1023	975	1223			
	1175	1154	1272			

TABLE 6. Women (continued).

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
K	1141	1138	1163			
	1116	1098	1194	—15·2	—16·0	—11·4
	1128	1118	1179			
L	1217	1163	1493			
	1222	1197	1330	—10·8	—13·7	+3·1
	1220	1180	1410			
M	1377	1400	1304			
	1294	1245	1223	—4·2	—5·2	—9·4
	1336	1322	1263			
N	1031	1031	1037			
	1407	1410	1412	—12·3	—12·5	—10·2
	1276	1260	1347			
	1238	1234	1266			
O	1409	1417	1388			
	1382	1385	1384	—10·6	—10·3	—11·3
	1396	1401	1386			
P	1409	1413	1407			
	1167	1157	1211	—17·1	—17·4	—15·4
	1069	1064	1097			
	1215	1211	1232			
Q	1333	1337	1328			
	1337	1357	1271	—7·2	—6·3	—9·6
	1335	1347	1299			
R	1344	1361	1294			
	1362	1368	1353	—13·4	—12·6	—15·4
	1353	1365	1323			
S	1236	1234	1254			
	1258	1201	1454	—7·0	—9·0	+0·7
	1272	1251	1373			
	1255	1229	1360			
T	1081	1070	1132			
	975	976	989	—33·8	—33·4	—35·9
	1112	1145	953			
	1056	1064	1024			
U	1145	1153	1123	—7·9	—7·2	—9·7

TABLE 6. Women (continued).

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
V	1487	1513	1401			
	1476	1499	1403			
	1291	1270	1378	—3·1	—2·5	—4·1
	1319	1322	1333			
	1393	1401	1379			
W	1066	1065	1082	—12·0	—11·8	—10·5
X	1046	1045	1059	—19·5	—19·5	—18·4
Y	1028	1128	1137			
	1070	1171	1174	—7·6	—7·5	—7·1
	1149	1150	1155			
Z	1407	1389	1503	—0·3	—1·5	+6·5
				—10·5	—10·9	—7·3

TABLE 7. Men.

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
1	1623	1624	1635			
	1677	1716	1428	+6·2	+7·2	—1·0
	1725	1753	1644			
	1675	1698	1569			
2	1155	1113	1323			
	1326	1270	1551			
	1091	1043	1320			
3	1508	1490	1590			
	1536	1533	1563	—13·3	—14·2	—9·3
	1486	1463	1584			
	1510	1495	1579			
4	1592	1528	1851			
	1630	1601	1752	—8·3	—9·8	—1·9
	1594	1609	1551			
	1605	1579	1718			
5	1624	1636	1593			
	1601	1609	1581	—10·5	—10·2	—10·6
	1507	1499	1548			
	1577	1581	1574			

TABLE 7. Men (continued).

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
6	1613	1604	1665	—14·4	—15·9	—12·7
8	1181	1179	1200			
	1183	1162	1275	—28·4	—29·2	—25·1
	1182	1170	1237			
9	1543	1522	1638	—3·3	—3·3	—1·9
	1522	1542	1461			
	1532	1532	1550			
10	1366	1330	1521			
	1486	1465	1578	—11·0	—13·4	—3·3
	1426	1387	1550			
11	1236	1208	1356	—18·0	—18·6	—14·0
	1286	1290	1281			
	1444	1435	1491			
	1322	1311	1376			
12	1364	1341	1464			
	1471	1457	1539	—12·1	—13·3	—6·8
	1418	1399	1502			
13	1347	1303	1527			
	1553	1520	1692	—11·1	—13·4	—1·4
	1425	1389	1575			
	1442	1404	1598			
14	1433	1436	1452	—12·6	—12·5	—11·5
15	1370	1346	1473			
	1416	1396	1500	—17·1	—18·4	—11·5
	1393	1370	1487			
16	1594	1569	1704			
	1496	1456	1665	—10·9	—12·3	—4·8
	1402	1395	1425			
	1497	1473	1598			
17	1515	1502	1574			
	1348	1304	1527	—14·8	—16·5	—7·7
	1432	1403	1550			
18	1464	1474	1443			
	1619	1615	1644	—8·8	—9·0	—7·0
	1540	1522	1626			
	1541	1537	1571			

TABLE 7. Men (continued).

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
19	1661	1637	1764			
	1704	1668	1851	—1·8	—3·1	+3·9
	1670	1663	1713			
	1678	1656	1776			
20	1734	1727	1776			
	1635	1626	1683			
	1606	1605	1626	—3·5	—3·2	—1·3
	1658	1653	1695			
21	1504	1488	1578			
	1541	1529	1635	—12·8	—13·6	—8·0
	1523	1508	1606			
22	1442	1408	1572			
	1631	1613	1711	—14·4	—16·1	—8·4
	1537	1515	1642			
23	1571	1580	1548			
	1442	1446	1455	—19·9	—20·3	—17·5
	1507	1472	1656			
	1507	1499	1553			
25	1460	1403	1689	—2·8	—5·7	+9·2
	1525	1492	1668			
	1493	1447	1678			
26	1652	1639	1713			
	1606	1599	1644	+4·2	+2·6	+6·4
	1640	1630	1695			
	1649	1623	1684			
28	1596	1594	1617			
	1682	1644	1800	—0·7	—1·2	+3·9
	1579	1561	1665			
	1619	1600	1694			
29	1401	1374	1518			
	1508	1533	1428	—12·9	—10·9	—9·9
	1375	1477	1491			
	1428	1461	1479			
30	1561	1513	1761	—5·5	—8·3	+6·7
31	1758	1744	1827			
	1763	1749	1854	+6·9	+6·2	+11·1
	1892	1884	1938			
	1804	1792	1873			

TABLE 7. Men (continued).

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
32	1622	1634	1590			
	1521	1518	1560	—9·1	—9·6	—6·1
	1485	1451	1629			
	1543	1534	1593			
33	1467	1457	1515			
	1362	1379	1311	—15·7	—16·1	—16·4
	1415	1418	1413			
34	1759	1762	1761			
	1609	1583	1722	—8·0	—8·6	—4·8
	1684	1672	1742			
36	2020	1990	1710			
	1901	1884	1575	+6·7	+5·4	—10·6
	2024	2002	1689			
	1982	1959	1658			
37	1695	1673	1794	—12·4	—13·5	—7·2
38	1385	1382	1398			
	1493	1507	1455	—17·3	—17·6	—15·4
	1213	1185	1329			
	1364	1358	1394			
39	1550	1573	1473			
	1584	1620	1458	—12·4	—11·2	—15·9
	1475	1482	1491			
	1536	1558	1474			
40	1447	1451	1446			
	1550	1550	1566	—14·6	—14·5	—14·1
	1498	1500	1506			
41	2065	2074	2055			
	2134	2156	2067	+15·0	+15·2	+15·4
	2115	2093	2220			
	2105	2108	2114			
42	1646	1636	1695			
	1545	1560	1503	+2·0	+2·1	+2·1
	1596	1598	1599			
43	1378	1338	1539			
	1322	1225	1425			
	1327	1284	1476	—14·5	—15·8	—8·1
	1251	1354	1236			
	1320	1300	1419			

TABLE 7. Men (continued).

Subject.	Basal Metabolism. Calories per 24 hours. Gasometer Method.	Oxygen Method.	CO_2 Method.	% Variation of Average from Du Bois. Gasometer Method.	Oxygen Method.	CO_2 Method.
44	1516	1538	1446			
	1508	1517	1485	—3·3	—2·4	—6·3
	1512	1527	1465			
45	1574	1567	1614			
	1600	1570	1728	+2·7	+1·5	+8·1
	1587	1569	1671			
46	1245	1228	1320	—29·4	—30·3	—25·1
47	1385	1353	1518			
	1524	1510	1593	+0·2	—0·7	+5·8
	1518	1515	1548			
	1476	1459	1553			
48	1703	1667	1854			
	1789	1800	1758	—0·1	—1·2	+2·9
	1746	1733	1806			
49	1226	1238	1197			
	1180	1191	1147			
	1188	1196	1174			
	1198	1208	1173	—22·4	—21·8	—24·1
50	1408	1397	1439	—17·7	—18·3	—15·8
				—8·9	—9·6	—6·0

TABLE 8. Subject X.

Time	Resp. Rate	Volume Expired 14.4 Minutes	Depth Respiration	R.Q.	Pulse Rate	CC per Minute CO_2	CC per Minute O_2	Metabolism Calories in 24 Hours Gasometer	Metabolism Calories in 24 Hours CO_2	Metabolism Calories in 24 Hours O_2	Type of Respiration
18-11-26:											
10.25–10.39·4	10	50·8	353	0·779	77	132	168	1158	1120	1167	Normal
11.5 –11.14·4	20	130·0	443	1·20	69	240	206	1494	2037	1432	Increased Rate and Depth
11.30–11.44·4	10	36·8	256	0·744	69	95	127	866	806	882	Normal
11.53–12.2	68	162·4	166	1·05	74	208	194	1409	1766	1348	Increased Rate
25-11-26:											
10.25–10.39·4	10	46·0	319	0·848	69	125	146	1020	1061	1015	Normal
10.55–11.9·4	10	99·5	691	1·19	70	224	187	1355	1900	1299	Increased Depth
11.40–11.54·4	10	45·6	317	0·788	65	122	154	1061	1036	1070	Normal
12.3 –12.17·4	30	90·0	210	0·954	65	160	166	1193	1358	1154	Increased Rate
30-11-26:											
10.32–10.46·4	9	44·7	345	0·841	67	126	148	1036	1067	1031	Normal
10.50–11.4·4	9	66·4	407	0·971	64	167	175	1261	1452	1214	Increased Inspiration
11.35–11.49·4	9	45·5	351	0·814	68	132	161	1118	1121	1120	Normal
11.51–12.5·4	10	94·5	656	1·09	72	212	192	1392	1840	1331	Increased Expiration
1-12-26:											
10.40–10.54·4	10	51·4	357	0·814	74	141	171	1189	1194	1191	Normal
11.5 –11.19·4	10	49·6	344	0·792	70	136	170	1174	1152	1182	Normal
11.30–11.44·4	10	81·3	565	0·949	70	187	196	1406	1591	36	Increased Inspiration

TABLE 9. Subject 50.

Time	Resp. Rate	Volume Expired 14.4 Minutes	Depth Respiration	R.Q.	Pulse Rate	CC per Minute CO_2	CC per Minute O_2	Metabolism Calories in 24 Hours Gasometer	Metabolism Calories in 24 Hours CO_2	Metabolism Calories in 24 Hours O_2	Type of Respiration
23-11-26:											
10.27–10.41·4	12	71·7	415	0·846	67	171	200	1399	1451	1390	Normal
11.39–11.53·4	11	95·0	600	0·851	60	189	212	1497	1605	1473	Increased Depth
12.6 –12.20·4	29	99·8	239	0·674	63	156	227	1534	1324	1577	Increased Rate
26-11-26:											
10.21–10.35·4	10	78·2	543	0·882	67	177	199	1403	1503	1383	Normal
10.45–10.55	10	145·8	1013	1·27	66	287	225	1634	2436	1564	Increased Depth
11.26–11.40·4	9	62·9	485	0·735	66	150	203	1376	1273	1411	Normal
11.49–12.2·4	28	110·9	275	0·800	62	192	238	1641	1630	1654	Increased Rate
2-12-26:											
10.15–10.29·4	10	76·9	534	0·889	58	178	199	1404	1511	1383	Normal
10.37–10·48	10	120·7	838	1·03	61	261	249	1810	2215	1730	Increased Inspiration
11.16–11.30·4	12	81·0	468	0·903	55	191	210	1488	1621	1459	Normal
11.38–11.50	12	127·0	735	1·11	61	273	244	1770	2318	1695	Increased Expiration

TABLE 10.

Effect of Depth of Respiration on B.M.R.

Rate of respiration.	% Increase in lung ventilation.	Alteration of R.Q.	% Increase in Basal Metabolic Rates. Gasometer Method.	Oxygen Method.	Carbon dioxide Method.
12–11	32%	·85–0·85	7%	6%	11%
9– 9	48%	·84–0·97	22%	18%	36%
9– 9	52%	·82–0·99	14%	10%	31%
12–12	56%	·90–1·11	19%	16%	43%
10–10	57%	·89–1·03	28%	25%	46%
10–10	64%	·79–0·95	20%	15%	32%
10–10	86%	·88–1·27	16%	13%	62%
9–10	107%	·81–1·09	25%	19%	64%
15–15	112%	·81–1·23	21%	16%	77%
10–10	116%	·85–1·19	33%	28%	79%
9– 9	170%	·80–1·27	40%	33%	111%

Effect of Rate of Respiration on B.M.R.

Rates of respiration.	% Increase in lung ventilation.	Alteration of R.Q.	Depth of Respiration.	% Increase in B.M.R. Gasometer Method.	Oxygen Method.	CO_2 Method.
12–29	39%	·85–0·67	415–239	10%	13%	—9%
9–28	76%	·74–0·80	485–275	19%	17%	28%
10–30	100%	·79–0·95	317–210	12%	8%	31%
12–25	118%	·83–1·22	458–479	8%	4%	53%
10–68	210%	·78–1·05	353–166	22%	15%	57%

TABLE 11. Subject X (7-3-27).

Time	Venous* Alveolar CO_2	Venous* Alveolar O_2	R.Q.	Volume Expired in Respiration	Volume Expired in 5 Minutes	Volume Expired in 14.4 Minutes	Depth Respiration CC	Metabolism Gasometer Method	Metabolism O_2 Method	Metabolism CO_2 Method	CC per 5 Minutes CO_2	CC per 5 Minutes O_2	Pulse Rate
10.40 a.m.	6·28	12·06											68
10.44–10.58·4			0·801	9	16·5	47·6	368	1017	1021	1009	594	735	68.68.68.68
11.0	6·15	13·04											
11.8–11.13			1·30	9	39·2	—	784	1367	1307	2088	1230	941	70
11.13½	4·43	16·46											
11.14–11.19			1·30	9	43·1	—	958	1341	1282	2064	1216	923	
11.19½	4·10	15·89											
11.20–11.25			1·21	9	50·8	—	1129	1572	1503	2241	1320	1082	70
11.25½	3·71	15·53											
11.8–11.23			1·27	9	44·3	127·8	956	1427	1364	2131	1255	982	

*Last portion of expired air after holding the breath 30 seconds.

TABLE 12. Subject 50 (3-3-27).

Time	Alveolar CO_2	Alveolar O_2	R.Q.	Volume Expired in Respiration	Volume Expired in 5 Minutes	Volume Expired in 14.4 Minutes	Depth Respiration CC	Calories per 24 Hours. Metabolism Gasometer Method	Metabolism O_2 Method	Metabolism CO_2 Method	CC per 5 Minutes CO_2	CC per 5 Minutes O_2
9.30 a.m.	5·49	14·41	0·800									
9.40–9.54·4			0·832	12	27·5	79·1	458	1485	1480	1520	896	1066
9.56	5·47	14·42	0·802									
10.10–10.15			1·22	25	55·9	—	457	1813	1732	2611	1538	1247
10.15–10.20			1·31	25	65·2	—	522	1532	1458	2390	1408	1054
10.20–10.25			1·13	25	58·3	—	466	1481	1417	1988	1171	1019
10.10–10.25			1·22	25	59·9	172·3	479	1609	1536	2330	1372	1107
10.25½	3·66	17·19	0·960									

TABLE 13. Subject X (9-3-27).

Time	Alveolar CO_2	Alveolar O_2	R.Q.	Volume Expired in Respiration	Volume Expired in 5 Minutes	Volume Expired in 14.4 Minutes	Depth Respiration CC	Metabolism Gasometer Method	Metabolism O_2 Method	Metabolism CO_2 Method	CC per 5 Minutes CO_2	CC per 5 Minutes O_2	Pulse Rate
9·35 a.m.	5·56	13·95	0·749										
9.41–9.54·4			0·822	9	16·6	47·8	369	1075	1074	1088	641	773	71.72.69
	Venous*												
9.56	6·40	11·72											
10.2–10.7			1·07	9	25·5	—	567	1236	1182	1557	917	850	70
10.7–10.12			0·98	9	25·5	—	567	1123	1079	1292	761	777	66
10.12–10.17			0·907	9·5	24·7	—	521	1320	1288	1428	841	928	68
10.2–10.17			0·986	9	25·2	72·7	551	1226	1183	1426	840	852	
	Venous*												
10.17	5·40	13·73											
10.19–10.29			0·705	9	14·7	42·3	326	1047	1080	940	554	776	69

*Last portion of expired air after holding the breath 30 seconds.

TABLE 14. Subject 50 (8-3-27).

Time	Alveolar CO_2	Alveolar O_2	R.Q.	Volume Expired in Respiration	Volume Expired in 5 Minutes	Volume Expired in 14.4 Minutes	Depth Respiration CC	Calories per 24 hours. Metabolism Gasometer Method	Metabolism O_2 Method	Metabolism CO_2 Method	CC per 5 Minutes CO_2	CC per 5 Minutes O_2	Pulse Rate
10 a.m.	4·66	15·53	0·823										56
10–10.15			0·805	15·5	30·0	86·6	439	1448	1453	1429	842	1046	53
	Venous*												
10.15½	6·10	11·7											
10.23–10.28			1·27	15·5	64·2	—	828	1931	1846	2865	1688	1329	58
10.28–10.32			1·26	15·5	66·1	—	853	1701	1625	2503	1474	1170	
10.33–10.38			1·16	15·5	61·5	—	794	1646	1572	2236	1317	1312	58
10.23–10.38			1·23	15·5	63·9	184·1	828	1759	1681	2535	1493	1210	
	Venous*												
10.38½	3·68	15·77											

*Last portion of expired air after holding breath 30 seconds.

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A NEW METHOD FOR THE PREPARATION OF BACTERIOLOGICAL CULTURE MEDIA CONTAINING AGAR

by

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(Submitted for publication 4th August, 1927.)

Perhaps among all classes of culture media used by bacteriologists there is none more widely employed than the series having agar-agar as a base. Yet despite its universality the methods of preparation have left much to be desired, the chief problem being its clarification, which was only accomplished satisfactorily to the detriment of the nutritive value of the media and with considerable loss of bulk.

It has been experimentally established that the lower the temperature of filtration of an agar medium, below 100°C., the less nutritive the resultant medium; also the less heating to which the medium has been subjected the higher the growth factor of the end product.

The following method was devised to give a clear medium from unwashed agar filtered above 100°C. There is little heating, but the method is simple, and gives uniform results, with little loss of nutritive value and bulk.

The method involves the making of two stock preparations, one a clear agar jelly, and the other a nutrient broth.

The clear agar jelly is prepared as follows:

Two pieces of fine gauze are cut about 1 foot square. From a roll of cotton wool a piece about 8 or 9 inches square and a few millimetres thick is carefully removed, taking care that there are no thin patches, and is laid between the pieces of gauze. The centre is then pushed down into a litre Erlenmeyer flask, and the required amount of agar, in a granular condition, is introduced. The pad is then formed into a bag, with the agar in the centre, and tied firmly with string at the mouth. The excess gauze is then cut off, and the bag suspended in the flask by means of the strings, which are conveniently held by a rubber band around the neck of the flask (*vide* figure).

Seven hundred and fifty c.c. of distilled water are then poured into the flask, and the bag allowed to float loosely in the water. It is now autoclaved for 25 minutes at 120°C. Allow to cool to 100°C., and remove from autoclove. Raise

the bag up towards the neck of the flask clear of the liquid, and replace in the autoclave for 10 minutes at 110°C. to allow all the agar to drain from the bag. When cool remove the empty bag from the flask, leaving the clear agar jelly.

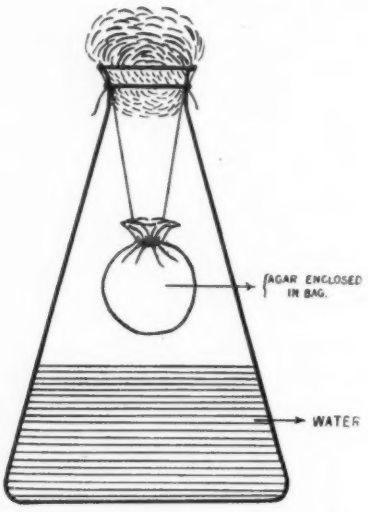

Figure 1.

The reaction may then be adjusted, and the nutrient broth, filtered and adjusted to the same reaction, added.

There is approximately 8% to 10% loss of agar, which may be allowed for, and a small loss of water, which can be made up where accurate measurements are required.

In actual practice, in making ordinary 2·5% nutrient agar the procedure is as follows:

For convenience in handling, the medium is made up in 1 litre lots. To make 1 litre of a 2·5% agar medium, 27 gm. of agar are used. This is dissolved in 750 c.c. of distilled water by the above method. The nutrient broth is prepared in quadruple strength by dissolving the meat extract (5 gm.), sodium-chloride (5 gm.), and peptone (10 gm.) in 250 c.c. of distilled water, heating for 20 minutes at 100°C., adjusting the reaction (to pH 7·3–7·4), and filtering through paper.

The 250 c.c. of quadruple strength broth are then added to the 750 c.c. of agar jelly, and the medium tubed and sterilized.

The agar jelly is conveniently prepared in batches of several flasks, each containing 750 c.c. These may be kept in stock, and when required for use 10 minutes at 120°C. is sufficient to liquefy the jelly.

OCCURRENCE OF *ONCHOCERCA GIBSONI* (WORM NODULE) IN CATTLE IN GIPPSLAND, VICTORIA

by

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(Veterinary Research Institute, Melbourne).

(Submitted for publication 15th November, 1927.)

Up to the present time it has been thought that cattle bred in Victoria were entirely free from infestation with this parasite; indeed, the parasite has not to my knowledge been reported from places further south than round about Eden, on the south coast of New South Wales.

A few months ago the occurrence of the parasite in cattle bred and reared in South Gippsland was brought to my notice by Mr. D. Poole, the Agricultural Department's stock inspector for this area, and with his guidance I was enabled to see several dairy herds in which one or more members were found to be infected on manual examination. From one of these cows a nodule lying in the subcutaneous tissue of the chest wall just behind the right elbow, and about the size of a walnut, was excised and examined. On section it presented the characteristic appearance, and sufficient of the parasite was dissected out for purposes of identification as of the *Onchocerca* genus.

A second visit was undertaken recently (November 9) by Dr. Georgina Sweet, Mr. George McLennan, B.V.Sc., Mr. D. Poole, and myself. A number of farms in the neighbourhood of Foster were visited, and plentiful evidence of the occurrence of worm nodules was obtained. On one dairy farm, with a herd of thirty animals, more than half were palpably affected.

So far as Mr. Poole's survey has gone, nodules have been found in native-bred and reared cattle along a strip of coast country centering on Foster, about 60 miles long, and going back inland for about 12 miles.

Further work is now being undertaken in the district by Mr. G. McLennan, with a view to determining the limits of the distribution, the biting flies prevalent in the area, and, if possible, experimental methods of transmission of the parasite.

FURTHER OBSERVATIONS ON THE LIFE HISTORY OF THE EYE WORM OF POULTRY

by

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(Submitted for publication 1st September, 1927.)

Fielding (1926) has shown that the Surinam cockroach, *Pycnocelus (Leucophaea) surinamensis* L., is responsible for the transmission of the eye worm *(Oxyspirura parvovum)* of poultry in Queensland. He stated that the cockroach picked up the larvae, but on further experimental work he has been unable to provide a continuity of larval life under cultural conditions. He has shown that when the infected cockroach is eaten up by the fowl, it does not pass beyond the crop before the contained worms liberate themselves, and pass up through the oesophagus into the mouth, and thence through the infra-ocular sinus or naso-lachrymal duct into the eyes, reaching their destination in a few minutes.

Influence of heat in the liberation of larvae.

Owing to the fact that the larvae take such a short time to reach the eyes, it was thought that the influencing factor in their liberation could not be due to any digestion of the roach, as this was practically nil. It was considered that possibly temperature was the main factor in their release, by simply providing a stimulus. To test this a few experiments were conducted by placing intact cockroaches in water heated to about 35°C. to 37°C., and it was seen that almost immediately the larvae began to pass through the thinner portions of the body, i.e., around the limbs, etc.

Influence of Humidity on Egg Laying.

From a series of daily examinations of the faeces and the mucous of the mouth it was noted that although a few eggs could be found on most days, there was a definite increase in their numbers on the approach of or during rain.

Hatching of the Eggs under External Conditions.

A series of experiments was conducted with the object of noting whether the eggs would hatch and the larvae live under controlled conditions. The media

used were variable. It was found that in some cultures a fairly large percentage hatched, and that in some cases hatching was delayed up to a maximum time of 25 days from the start of the experiment. Soon after obtaining their freedom, however, the larvae became quiescent, and on keeping them under constant observation no movement was noted; it was consequently decided they were dead. This series was combined with observations on egg laying, longevity of the females, etc. The results are enumerated in Table 1.

TABLE 1.

Series.	Cultural Solution.	Number of adult females used.	Longevity of females.	Eggs passed on days.	Living larvae seen on days.	Remarks.
A	Water	14	Few hours	—	—	Adults burst. Dead larvae seen on 4th, 6th, 13th, and 17th day
B	0.9% Sod. Chloride	21	2–6 days	2nd–7th	5th–25th	In some experiments no larvae were seen
C	1.4% Sod. Chloride	4	3–8 days	2nd onward	8th–15th	In two experiments no larvae seen
D	0.9% Sod. Chloride + Eye Fluid	2	4 days	2nd–3rd	9th	
E	0.9% Sod. Chloride + Eye Fluid 1 drop to each c.c.	11	3–6 days	2nd onward	11th–17th	In two cultures the worms were teased. In three cultures no larvae were seen. In one culture no eggs were passed
F	1.0% Sod. Chloride 0.5% Sod. Phosphate 0.1% Sod. Bicarbonate in Aqua dist.	5	3–5 days	3rd onward	4th–11th	In two cultures no larvae were seen. In two cultures the worms were teased
G	1% Citrated fowl blood	3	3 days	2nd	5th	In one culture no eggs were passed
H	Earth moistened with water	5	Few hours to 5 days	4th	3rd	In four cultures the adult burst, liberated eggs showed larvae on 3rd day. Other adult passed eggs on 4th day but showed no development
I	Fowl faeces+Sand+Saline	12	Broken up	—	3rd–4th	The cultures were made of egg free faeces sterilized
J	Sterile fowl faeces, Saline and Charcoal	10	Broken up	—	5th & 7th	In three cultures no larvae were noted
K	Bread moistened with Saline	9	Broken up	—	3rd	Larvae in two experiments only, eggs seen in other

Free Larvae in the Oviduct of the Female.

The occurrence of larvae free in the oviduct was noted on a number of occasions while conducting the series tabulated below. It was interesting to note that although the females concerned were frequently laying eggs in the cultures, these larvae (in some cases) did not pass out, but remained alive in the oviduct for varying periods up to a maximum, in one female, of 5 days. In one case the larvae were alive on the death of the parent worm.

Experiments with Guinea-pigs.

In view of the finding that the development of larval nematodes on gaining access to an accidental host is generally arrested, and the third stage larvae remain in this stage, and proceed to re-encapsulate (Seurat), it was considered of interest to test this by introducing the eye worms to a guinea-pig, and at the same time to note whether they would find their way into the eye, and also, when deliberately introduced into the eye, whether develcpment was arrested or not.

Experiment 1. A large number of eye worms (40 to 50) in the third stage were placed in the mouth of a guinea-pig, which was kept under observation for six weeks, but no evidence of worms in the eyes or re-encapsulation was found.

Experiment 2. Owing to the smallness of the eye cavity, it was decided to use only a limited number of the worms (about 12), which were placed in the left eye; thereafter the worms were extracted at varying intervals for the purpose of noting whether development kept pace with worms in their normal habitat. It was found that the parasites in this situation developed normally (so far as the experiment went), but apparently they could not find their way from one eye to the other, as none were seen except in the original location. The measurements, which compared favourably with those from the chicks and ducklings, are given in Table 2.

TABLE 2.

Day.	No. of Worms.	Measurements in mm.	Remarks.
6th	3	6·55, 6·65 & 7·37 x 0·19 to 0·24	Worms moulted.
8th	2	6·18 & 6·37 x 0·19	
12th	3	5·28, 6·8 & 7·0 x 0·18 to 0·22	
16th	2	9·24 & 8·84 x 0·24	Apparently moulting.
26th	1	8·22 x 0·24	Development advanced.

Preliminary to Experimental Infection of Cockroaches.

Since the majority of local poultry yards are infected with eye worms, and in consequence of a very large percentage of the cockroaches being infected, it became necessary to find an uninfected yard with plenty of cockroaches. This was difficult, since yards uninfected with eye worms were usually almost barren

of cockroaches. About 800 roaches were examined from different parts of the town, but batch after batch had to be discarded owing to infection. After some weeks of patient work examining the body cavity for the presence of capsules and larvae, and the alimentary tract microscopically for the presence of eggs or first stage larvae, a batch was obtained which showed no infection in about 100 cockroaches examined, and it was considered that this lot could be used for the purpose of experimental infection.

Experimental Observations on Infection.

Experiment 3 was carried out with the object of ascertaining the time taken from the ingestion of the eggs of the worm to the time the larvae are infective. For this purpose a large batch of cockroaches were starved for three days. They were segregated in a large museum jar containing sterilized earth and a few dried leaves for hiding-places; bread soaked with physiological salt solution containing a large number of eggs, which had been taken from teased female worms, was then introduced into the jar. The bread was noted on the third day to contain a few living larvae, and was all eaten up by the fourth day. The results of examinations are tabulated below.

Experiment Started. Day.		
1st.	Nymph and female examined.	Fully and under-developed eggs seen in gut.
	Bread examined.	Only eggs observed.
2nd.	Bread examined.	Only eggs observed.
3rd.	Nymphs and males examined.	Eggs and larvae measuring 235–260μ in length and 12–13μ in diameter seen in gut.
	Bread examined.	Eggs and larvae similar to above.
4th.	Bread consumed.	
6th.	Nymphs examined.	Eggs, uncapped eggs, and larvae seen in gut, one larva just emerging from egg-shell, which took 3 hours to get clear; this larvae measured 250 x 13μ. No larvae seen in body cavity.
10th.	Nymph and female examined.	The nymph showed larvae in gut measuring 235–250μ by 12–14μ. The female showed larvae free in body cavity, measuring 327–360μ by 18–20μ.
13th.	Nymphs examined.	No larvae found in gut, but larvae measuring 320–360μ by 18–20μ were observed in body cavity.

Experiment Started. Day.		
17th.	Male, female, and nymphs examined.	Capsules measuring 273 by 364μ in diameter; the contained larvae, measuring from 485 to 600μ by 26 to 28μ, were quiescent.
18th.	Nymphs examined.	Capsules and worms similar to above. Larvae quiescent.
25th.	Nymphs examined.	Capsules found measuring 295 to 309μ by 360 to 436μ; the contained worms measured 0·63 mm. to 1·01 mm. long by 0·028 mm. to 0·035 mm. in diameter.
32nd.	Nymphs examined.	Capsules found containing larvae measuring 1·9 mm. to 2 mm. long by 0·07 mm. to 0·08 mm. in diameter.
39th.	Nymphs examined.	Capsules found containing worms measuring 3·4 mm. to 3·8 mm. long by 0·118 mm. to 0·127 mm. in diameter. (See Experiment 4.)
46th.	Nymphs examined.	Capsules found containing worms measuring 4·6 mm. to 4·9 mm. long by 0·127 mm. to 0·145 mm. in diameter. (See Experiment 5.)
52nd.	Nymphs examined.	Capsules found, worms measured 4 mm. to 5·3 mm. long by 0·127 mm. to 0·145 mm. in diameter. (See Experiments 6, 7, and 8.)

Attention is here drawn to the occurrence of two frequently, and sometimes three, larvae in the one capsule.

Experiment 4. Chick one week old given a large number of capsules from roaches which had been fed with bread containing eggs 39 days previously. No eye worms were discovered in the eyes of the chick. Result negative.

Experiment 5. Broken roaches (infected 46 days previously), containing large number of capsules, given to a two weeks' old chick. Result negative.

Experiment 6. Chick three weeks old given 2 nymphs (infected 52 days previously), containing a large number of capsules. Worms observed on the tongue, roof and floor of mouth 4 minutes afterwards, but were not seen in the eyes until 45 minutes had elapsed. Result positive.

Experiment 7. Similar to above, with 3 nymphs. Worms in the mouth 4 minutes after introduction and inside of the eyes after 6 minutes. Result positive.

Experiment 8. Chick three weeks old given four unbroken roaches after anaesthetizing (infected 52 days previously). Worms were not observed in the mouth, but were seen passing through the naso-lachrymal duct into the eyes 20 minutes after introduction. Result positive.

Control chick to above experiments remained negative throughout.

Observations on Infection under Natural Conditions.

A young infected rooster which had been used for a previous experiment was introduced into a pen in which no infected fowls had been kept and no infected cockroaches had been found after a long series of prior examinations. Roaches were then examined from this pen at frequent intervals, and after 61 days capsules and worms from these roaches were given to a chick (Experiment 9). Worms were found in the eyes 15 minutes afterwards. Result positive after 61 days.

Experiment 10 to note whether the Worms are Infective to Pigeons.

A full-grown pigeon was given two cockroaches, one of which was dead, but contained a large number of capsules and free worms; the other roach was killed and opened to ascertain the presence of capsules. Eight minutes after introduction the worms were in the eyes. Result positive.

TABLE 3.

Observations on the Worms in the Cockroaches.

Days after Feeding.	No. of Worms.	Location.	Measurements. Length.	Diameter.	Remarks.
3rd-10th	Many	Free in gut	245–260μ	12–14μ	1st stage larvae.
10th-14th	Many	Free in body cavity	320–360μ	18–20μ	1st stage larvae.
17th-18th	Few	In capsules	485–600μ	26–28μ	1st stage larvae.
25th	Few	In capsules	0·63–1·01mm.	0·028–0·035mm.	Moulting
32nd	2	In capsules	1·91–2·09mm.	0·073–0·082mm.	2nd stage larvae.
39th	4	In capsules	(1) 3·5mm.	0·127mm.	2nd stage larvae.
			(2) 3·8mm.	0·118mm.	
			(3) 3·7mm.	0·118mm.	
			(4) 3·4mm.	0·118mm.	
46th	4	In capsules	(1) 4·9mm.	0·145mm.	Moulting
			(2) 4·9mm.	0·145mm.	
			(3) 4·8mm.	0·136mm.	
			(4) 4·6mm.	0·127mm.	

Days after Feeding.	No. of Worms.	Location.	Measurements. Length.	Diameter.	Remarks.
52nd	4	In capsules	(1) 5·3mm.	0·09mm.	3rd stage
			(2) 4·0mm.	0·1mm.	larvae.
			(3) 5·1mm.	0·09mm.	
			(4) 5·09mm.	0·1mm.	

Observations on the Worms from Introduction to Fowl to Egg Laying of Female.

This series was intended to supply information regarding the measurements at the different stages in continuation of the previous series; time taken from introduction of larvae to definitive host, to maturity, and egg-laying of females. For this purpose the experimental chicks and ducklings were used as reservoirs, from which the worms were withdrawn at varying intervals. The results are tabulated in Table 4.

TABLE 4.

Days after Introduction.	Number of Worms.	Source.	Measurements. Length in mm.	Diameter in m.m.	Remarks.
1	1	Duckling	5·3	0·182	Moulting.
1½	1	Duckling	5·1	0·16	Moulting.
2	1	Duckling	5·5	0·136	Moulting.
3	1	Duckling	5·64	0·109	Moulted = 4th stage larva.
4½	1	Duckling	5·9	0·14	Buccal cavity increasing in size.
6	3	Duckling	5·22	0·14	Muscular oesophagus distinct.
			4·55	0·127	
			5·1	0·14	
7	1	Duckling	5·46	0·145	
8	3	Duckling	5·27	0·127	
			5·49	0·145	
			5·46	0·127	
9	2	Duckling	5·5	0·127	
			5·3	0·118	
12	5	Ducklings (2) Chicks (3)	5·5	0·145	Length of oesophagus, 0·819 mm.
			6·18	0·182	
			7·09	0·182	Distance to nerve ring, 0·182 mm.
			8·3	0·23	
			8·46	0·218	

Days after Introduction.	Number of Worms.	Source.	Measurements. Length in mm.	Diameter in mm.	Remarks.
15	4	Duckling (1)	8·5	0·25	
		Chicks (3)	9·0	0·25	
			9·19	0·24	Moulting.
			7·18	0·24	
19	1	Chick	7·8	0·27	
26	2	Chick	5·8	0·1	
			9·0	0·24	
28	1	Chick	8·2	0·218	
38	2	Chick	4·9	0·127	
			7·6	0·25	Egg laying noted.

Attention is directed to the size of No. 1 worm in each case on the 26th and 38th days. These worms were obviously under-developed.

Distribution of Oxyspirura parvovum, O. mansoni, and Pycnocelus (Leucophaea) surinamensis.

That there is an intimate association between the known distribution of the eye worm, *Oxyspirura parvovum*, and the cockroach. *Pycnocelus (Leucophaea) surinamensis* in Australia has recently been pointed out by Fielding. Both vector and parasite are known to occur at Townsville, Cairns, Ayr, and Hughenden. Messrs. Conigrave and Pugh informed the writer that the worm occurs at Darwin, the cockroach having been recorded previously from that locality.

It would appear also that the association between *O. mansoni* and the Surinam cockroach holds good, since both occur in China, Java, Brazil, and Honolulu. In Rabaul the eye worm occurs; and in New Ireland, which is closely adjacent, the cockroach has been recorded. This association is further accentuated by the fact that workers in Florida have recently found a cockroach implicated in the transmission of *O. mansoni*, which is regarded as the same species as the Australian vector.

SUMMARY OF CONCLUSIONS.

Although living larvae have been detected in the oviduct of the female worms (in which position they can live for some days), and notwithstanding the fact that living larvae have been noted up to a maximum of 25 days in culture, it would appear that there is no continuity of larval life under experimental conditions. It is now considered that normally the eggs hatch in the gut of the cockroaches. One larva was observed emerging from the egg two days after

the last possible ingestion of eggs by the insect; larvae were noted in the gut six days after the last possible ingestion of eggs, and were found in the body cavity ten to thirteen days after the initial feed, or six to nine days after the last ingestion of eggs. Seventeen days after the initial feed, encapsulated larvae were found, which were approximately twice the size of newly-hatched larvae. It was found that the larvae were not infective 46 days after experimental feeding of the insect, but are so 52 days after, and that the female begins laying eggs 38 days after entering the definitive host, thus making the cycle of the parasite complete in approximately 13 weeks, though under more favourable conditions this time may probably be somewhat reduced. It was found also that on introducing infected cockroaches to pigeons the latter became infected, and that worms introduced into the eye of a guinea-pig were capable of continuing their development, but when introduced into the mouth of this animal they were incapable of reaching the eye.

Lastly, from the known distribution of the eye worms, *O. parvovum*, *O. mansoni*, and the cockroach *Pycnocelus (Leucophaea) surinamensis*, it would appear that there is a direct association between them.

ACKNOWLEDGMENTS.

In conclusion, I have to acknowledge my indebtedness to the Directors of the various Australian Museums, and Mr. Gurney (Government Entomologist of New South Wales), for supplying me with information regarding the distribution of the cockroach. I have also to thank Dr. A. H. Baldwin and Dr. G. M. Heydon for advice and assistance during the progress of this research.

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IN VITRO TESTS OF THE TOXICITY OF CERTAIN DRUGS FOR HYDATID SCOLICES

by

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(Submitted for publication 1st September, 1927.)

It has long been hoped that some method of treating hydatid disease of man, other than by surgical intervention, might be found, and from time to time claims have been made that certain drugs and also X-rays have been found effective as curative agents. Thus the use of Filix-mas, Kamala, tartar emetic, emetine, and arsenobenzol has been advocated. It cannot be stated that the evidence adduced in support of these claims has been such that conclusions of any value could be drawn from them. Moreover, Dévé (4, 5) has shown that in rabbits experimentally infested with hydatid cysts by the subcutaneous injection of hydatid "sand," the administration of large doses of Filix-mas and Kamala did not destroy the parasite or markedly inhibit its growth. In the same way Dévé and Payenneville (7, 8) found neosalvarsan and novarsenobenzol to be equally ineffective, while Dévé (3) and Dévé and Billard (6) found that hydatid scolices exposed to the action of X-rays, either before or after injection into the rabbit, were still capable of growth and cyst formation. Later, however, Dévé (5) obtained some indication that the vitality and regenerative powers of scolices exposed to X-rays of 20,000 R were markedly affected, so that he considers that further work along these lines is indicated.

The only controlled test of value in man was that of Fairley (9), who in two cases of hydatid disease injected amounts of tartar emetic approximating those administered in schistosome infestation. The drug apparently had no effect, as shown by subsequent operation in one case and complement fixation tests in the other, while in the former no trace of the drug could be recovered in the hydatid fluid. In spite of these observations the subject was considered to be worthy of further experimental investigation. It was decided to test the action of certain drugs on hydatid cysts *in vivo*, and as a preliminary *in vitro* tests on hydatid scolices were conducted, the results of which are embodied in this paper.

It was decided only to test drugs which are capable of being injected *in vivo* in relatively large doses, and which from their action on other parasitic infestations offer some likelihood of proving efficacious. The drugs selected were

trypan blue, the value of which as a curative agent in various protozoan affections is established; acriflavine as representative of a different group of dyes, and because of its highly potent bactericidal action, at least *in vitro;* tartar emetic, whose toxicity for certain metazoan parasites is well proven, and of which another example has recently been provided by Itaki and Makino (11), who found it to have a marked effect upon the larvae of the *Dirofilaria immitis*, the heart-worm of the dog; and emetine, which again is largely used as a parasiticide, and which, moreover, was considered by Cawston (1), when used in conjunction with tartar emetic to have inhibited the growth of an hydatid cyst in man.

METHOD OF EXPERIMENTATION.

Hydatid scolices were obtained from hepatic and pulmonary cysts in the sheep, being drawn off under aseptic precautions. The cysts were first freed from the surrounding tissues, washed in alcohol, and the wall was then seared and pierced by a red-hot needle. After allowing a small quantity of the fluid to escape, and so wash out any contaminants, the contents were run into sterile containers. Approximately equal quantities of brood capsules and scolices were then added to varying dilutions of each drug, and incubated at 35°C. The drugs in all cases were dissolved in normal saline. Controls of scolices in normal saline and hydatid fluid were also cultured at 35°C., and were kept under observation for 48 hours, during which time a large majority remained alive and active.

The determination of the lethal time was made by pipetting off small quantities of scolices at stated intervals, and observing these in cavity slides on a warmed stage at 39°C. Those which had died were readily demonstrable by the loss of clarity of the various structures, such as the definition of the cuticle, and the dulling of the calcareous corpuscles and hooks, and more especially by the cessation of the active contractile movements seen in normal scolices.

ACTION OF DRUGS TESTED.

1. *Acriflavine.* The dilutions of the drug used were 1–200, 1–1,000, 1–2,000, 1–5,000, 1–10,000, 1–100,000.

After one hour's exposure to the action of the drug large numbers of scolices in 1–200 solution were dead, all others being active. After two hours all were dead in 1–200 solution, while in the other solutions the majority were alive. After three hours all were dead in 1–1,000 and many in 1–2,000 solutions. In four hours all scolices in 1–2,000 were dead, in six hours all in 1–5,000, and in eight hours all in 1–10,000. After eighteen hours the majority of the scolices in 1–100,000 were dead, but an occasional scolex was still moving feebly.

2. *Trypan Blue.* Dilutions of the drug used were 1–200, 1–1,000, 1–2,000, 1–5,000.

After one hour's exposure all scolices were alive, but few had evaginated. After two hours all scolices were alive, and about 25% in 1–200 solution had evaginated. In four hours all were alive, and the majority in 1–200 solution were evaginated, and a considerable proportion in 1–1,000. After eight hours all scolices were still contracting actively, and a large proportion in all solutions had evaginated. In sixteen hours almost all scolices in 1–200 solution were dead, but an occasional scolex was still moving, many were dead in 1–1,000 solution, but the majority were alive in 1–2,000 and 1–5,000 solutions. After twenty-four hours an occasional scolex still showed movement in 1–1,000 solution, while about 20% were alive in 1–2,000 and 1–5,000 solutions.

Figure 1. Scolices in Acriflavine, 1–5,000, after five hours' exposure, showing "spherules belleuses."

3. *Tartar Emetic.* The dilutions of the drug used were 1–100, 1–1,000, 1–2,000.

After two hours all scolices were alive and moving actively, and few were evaginated. In three hours many scolices in 1–100 solution were dead, those in the other solutions being alive. After four hours all in 1–100 were dead, while those in 1–1,000 were commencing to die, few of these scolices having evaginated. In six hours all scolices in the 1–1,000 solution were dead, as were a few in 1–2,000. In eight hours the majority in 1–2,000 were dead, while in ten hours all were dead. Only a small proportion of scolices in the various dilutions of the drug evaginated.

4. *Emetine.* Dilutions of the drug used were 1–100, 1–1,000.

After four hours all scolices were alive. After six hours a few scolices in 1–100 solution were dead, and in eight hours all scolices in this solution were

dead. After fourteen hours the majority of the scolices in 1–1,000 solution were alive. Few scolices in either solution were evaginated.

Consideration of the above results reveals a striking variation in the action of the drugs on hydatid scolices. Acriflavine proved most toxic, a 1–1,000 solution being fatal in three hours, a 1–10,000 solution in eight hours, while even 1–100,000 killed the great majority of scolices in seventeen hours. When compared with that of acriflavine the action of trypan blue is surprising, scolices remaining alive and active in a 1–200 solution for sixteen hours, while after twenty-four hours an occasional scolex in 1–1,000 solution was alive, and a considerable proportion in 1–2,000. After acriflavine, tartar emetic was most rapidly fatal, a 1–1,000 solution killing all scolices in six hours. Emetine was relatively ineffective, a large proportion of scolices being alive and actively contracting after fourteen hours in a 1–1,000 solution.

As was to be expected, none of the drugs gave results comparable with those given by corrosive sublimate or formalin, which in a dilution of 1–1,000 and 1–200 respectively were found by Coutelen (2) to be fatal in two to three minutes.

It is not considered that the results obtained necessarily indicate the relative potency of the drugs *in vivo*, since, though acriflavine has a relatively high bactericidal potency *in vitro*, and might be considered to be equally valuable *in vivo*, doubt has been cast on its ability to inhibit bacterial growth *in vivo* by Meleny and Zau (12). On the other hand, Fairley (10) has shown that the toxicity of tartar emetic for schistosome cercariae is immeasurably increased in serum, and therefore probably *in vivo*, when compared with its action in aqueous solution *in vitro*. Moreover, it cannot be determined how far length of exposure to very dilute solutions of the drugs *in vivo*, as in a long course of administration in hydatid disease might not produce results at variance from those obtained after a relatively short exposure to high concentrations, as in the above experiments.

Other Phenomena Observed. As was also found by Coutelen (2) bacterial contamination is markedly deleterious to the existence of hydatid scolices *in vitro*, since when this is heavy death may take place in less than 24 hours, and no scolices are seen to survive for longer than 48 hours. Evagination of scolices appeared to be influenced to a considerable extent by the action of the drugs. Thus in the case of acriflavine it appeared that death in many cases took place so rapidly that only a small minority evaginated, as shown by the figure, while in trypan blue the process seemed to be hastened, the great majority evaginating in its dilutions before those in the control cultures. In emetine, on the other hand, a large majority of the scolices remained invaginated, though alive, after fourteen hours. Another indication of the toxic action of the drug, and the approaching death of the scolices was the detachment of the scolices from the

everted wall of the brood capsules. In a few hours, in the higher strength acriflavine solutions, practically all were detached, while in trypan blue the great majority remained firmly attached by their pedicels to the wall.

Figure 2. Scolices in trypan blue, 1–1,000, after six hours' exposure.

It was also observed that many scolices appeared to emit bubble-like objects from either the anterior pole or that at which the pedicel is attached. These without doubt are the "spherules bulleuses" described by Coutelen (2), of which the exact nature is not clear. Coutelen considers they may be either thrown out as a result of the change from the high intracystic pressure to that of the external *milieu*, which is probably less than the internal pressure of these scolices, or be an indication of the commencing degeneration of the scolex. From my observations the latter would appear to be the case, since their formation was seen to occur most numerously shortly before death. In acriflavine solution, 1–2,000, they would be observed in from two to three hours, while in trypan blue solutions they would not appear till very much later, and then only shortly before death. The bubbles were clear and structureless, and it was not possible to see the fine granulations which Coutelen has at times found.

SUMMARY.

The action of acriflavine, trypan blue, tartar emetic, and emetine on hydatid scolices was tested *in vitro*. Acriflavine proved most highly toxic, and then tartar emetic, emetine, and trypan blue in the order named.

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